Designed, Synthetically Accessible Bryostatin Analogues Potently Induce Activation of Latent HIV Reservoirs *in vitro*

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Supporting Information

Contents:

General Synthetic Methods and Apparatus

Unless otherwise noted, all reactions were run under a nitrogen atmosphere in flame or oven dried glassware. Reactions were stirred using Teflon-coated magnetic stirrer bars. Reactions were monitored using thin layer silica gel chromatography (TLC) using 0.25 mm silica gel 60F plates with fluorescent indicator from Merck. Plates were visualized by treatment with UV, acidic *p*-anisaldehyde stain, or $KMnO_4$ stain with gentle heating. Products were purified via column chromatography using the solvent systems indicated. Silica gel 60, 230-400 mesh, was purchased from EM.

When necessary, solvents and reagents were purified before use. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under N_2. Ethyl ether (Et_2O) and dichloromethane (CH_2Cl_2) were passed through an alumina drying column (*Solv-Tek Inc.*) using nitrogen pressure. Toluene was dried by either of the aforementioned methods. Anhydrous dimethylformamide (DMF) and dimethyl sufoxide (DMSO) were obtained from Acros Organics. Ethyl acetate (EtOAc), petroleum ether, pentane, hexanes, and methanol (MeOH) were obtained from Fisher Scientific. DMSO used in bioassays and used to prepare biological samples was obtained from Fisher BioReagents (Class III). Powdered 4Å molecular sieves (< 5 micron) were purchased from Aldrich and stored/activated as indicated. Amine bases (Et_3N, pyridine, diisopropylamine) were distilled from CaH_2 under nitrogen. All other reagents were purchased from commercial suppliers (Aldrich, Acros, Strem) and were either used as received without additional purification or were purified using standard methods. ^{3}H-phorbol dibutyrate (^{3}H-PDBu) was obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO) as a solution in acetone with a specific activity of 20 μCi/mmol. Samples prepared for biological evaluation were purified via preparative HPLC in a water/acetonitrile (MeCN) gradient using a Varian Pro-Star (model 320) system equipped with an AllTech Alltima C18 column (10 μm, 10 x 250 mm).

NMR spectra were measured on a Varian INOVA 500 (^{1}H at 500 MHz, ^{13}C at 125 MHz), a Varian 400 (^{1}H at 400 MHz, ^{13}C at 100 MHz), or a Varian INOVA 600 MHz (^{1}H at 500 MHz, ^{13}C at 150 MHz) magnetic resonance spectrometer, as noted. ^{1}H chemical shifts are reported relative to the residual solvent peak (chloroform = 7.26 ppm; benzene = 7.15 ppm)[1] as follows: chemical shift (δ), (multiplicity (s = singlet, bs = broad singlet, d = doublet, bd = broad doublet, t = triplet, q = quartet, p = pentet), integration, coupling constant(s) in Hz, proton ID [when available, designated by carbon number]). ^{13}C chemical shifts are reported relative to the residual deuterated solvent ^{13}C signals ($CDCl_3$ = 77.16 ppm, C_6D_6 = 128.06 ppm)[1]. nOe's are reported in percent, relative to the intensity of the irradiated signal, set at -100%. ROESY enhancements are reported in percent, relative to the enhancement observed for geminal protons at +100%. Infrared spectra were recorded on a Perkin-Elmer 1600 Series Fourier transform spectrometer (FTIR) and are reported in wavenumbers (cm^{-1}). Optical rotation data were obtained using a JASCO DIP are reported as $[\alpha]_D^T$ (*c* = grams/100 mL), where D indicates the sodium D line (589 nm) and T indicates temperature (when noted, all optical rotation values were obtained at ambient temperature, ca. 22 – 25 °C). Unless otherwise indicated, optical rotations are the average (± standard deviation) of 10 individual measurements. High resolution mass spectra were obtained at the Vincent Coates Mass Spectrometry Laboratory, Stanford, CA, 94305.

[1] Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512-7515.

PKC Binding Assay Protocol

The protein kinase C (PKC) binding affinity of test compounds was performed via competition with ^{3}H-phorbol-12,13-dibutyrate (^{3}H-PDBu) as described below. This entails a glass-fiber filtration method to determine bound radioligand.

A. Preparation of the binding assay buffer

To a polypropylene vial was added Tris-HCl (pH 7.4, 1 M, 1 mL), KCl (1 M, 2 mL), $CaCl_2$ (0.1 M, 30 μL), and bovine serum albumin (40 mg, from Sigma). This mixture was diluted to 20 mL with deionized H_2O. The buffer was stored on ice until use. The final concentration of these constituents are shown in Table 6.1.

PKC binding assay buffer composition

Constituent	*Stock concentration*	*Quantity*	*Final Concentration*
pH 7.4 Tris-HCl	1.0 M	1.0 mL	50 mM
KCl	1.0 M	2.0 mL	100 mM
$CaCl_2$	0.10 M	30 μL	0.15 mM
Bovine Serum Albumin	-	40 mg	2 mg/mL
Deionized H_2O	-	To 20 mL final volume	-

B. Preparation of phosphatidyl serine (PS) vesicles and PKC mixture

For each compound to be tested, 1.75 mg phosphatidyl serine (Avanti Polar Lipids, porcine, obtained as a solution in $CHCl_3$) was isolated by removing chloroform. The solid PS was suspended as vesicles in binding assay buffer (1.75 mL) by sonicating (Branson Sonifier 250, power = 6, 40% duty cycle) four times for 30 s with a 30 s rest between sonications. The resulting cloudy mixture (1 mg/mL PS) was stored on ice until use.

Assay Protein Kinase C (PKC) was prepared by dissolving an aliquot of a rat-brain PKC isozyme mixture (stored at -20 °C in 50% glycerol)[2] in binding assay buffer solution (5.8 mL final volume for each compound to be tested) on ice. The quantity of concentrated PKC mixture used was batch-dependent; sufficient PKC was used so that assay signal to noise was roughly 10:1. The diluted PKC was stored on ice for immediate use.

C. Preparation of compound and ^{3}H-PDBu dilutions

^{3}H-PDBu (American Radiolabeled Chemicals, Inc.; specific activity: 20 μCi/mmol) was diluted 10-fold in DMSO from a 1 mCi/mL commercial acetone solution. This 500 nM stock solution was further diluted in DMSO to 30 nM for use in assays. The 500 nM and 30 nM stock solutions were stored in frozen DMSO

[2] A PKC isozyme mixture is employed, isolated by the method of Mochly-Rosen, see: Mochly-Rosen, D.; Koshland, Jr., D. E. *J. Biol. Chem.* **1987**, *262*, 2291-2297.

at −20 ºC until use. Compound dilutions were also prepared in DMSO, serially diluting from a chosen high concentration (depending on analog potency) by factors of 3 to 5. Seven analog concentrations were used to define the inhibition curve.

D. Assay protocol

Triplicate data points were obtained for each analog concentration. For each data point, phosphatidylserine vesicles (60 µL of 1 mg/mL), diluted PKC (200 µL), and diluted test compound (20 µL) were added to a polypropylene vial. ^{3}H-PDBu (30 nM in DMSO, 20 µL) was then added to all vials. Non-specific ^{3}H-PDBu binding was assessed in triplicate by substitution of test compound with unlabeled PDBu (20 µL of a 75 µM stock, assay concentration: 5 µM) or a similarly potent unlabeled PKC ligand. Maximal ^{3}H-PDBu binding was analyzed in triplicate by substitution of test compound with 20 µL DMSO. The solutions were mixed via vortexer, incubated at 37 °C for 5 min, and incubated on ice for at least 15 min prior to filtration.

Glass-fiber filters (Whatman GF/B, 21 mm) were prepared by soaking in a solution of aqueous polyethyleneimine (10% by volume, 6 mL) diluted in water (200 mL) for ≥60 min. Rinsing buffer (500 mL, 20 mM Tris, pH 7.4) was cooled on ice for the duration of the incubation period and for the remainder of the assay.

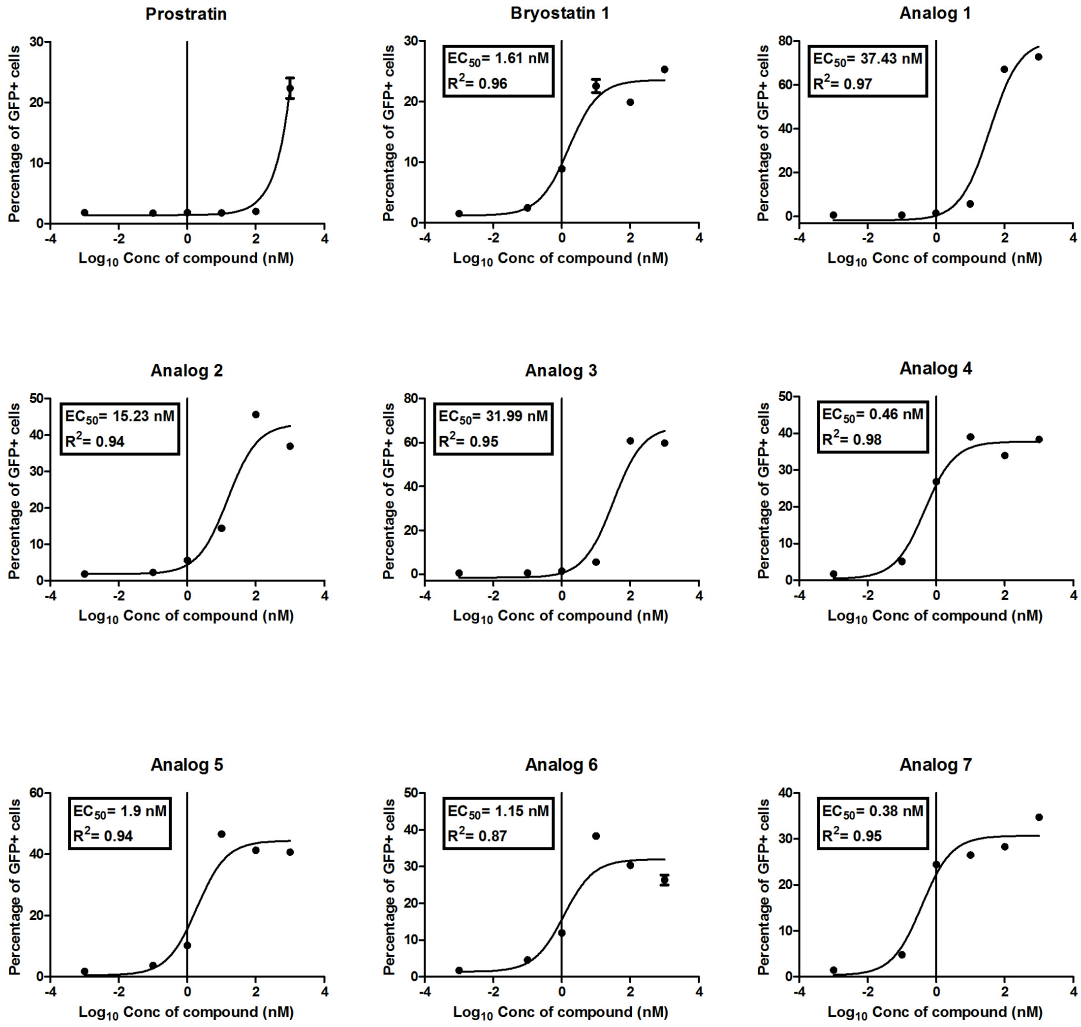
Assay vial contents were vacuum-filtered through the polyethylenimine-soaked filters, washing residual vial contents with 0.5 mL ice-cold rinse buffer. The filters were then washed dropwise with ice-cold buffer (4.5 mL), allowed to partially dry (~15 s) on the filter apparatus, and placed into scintillation vials. Scintillation vials were filled with Bio-Safe scintillation fluid (5 mL) and were measured for radioactivity using a Beckman LS 6000SC scintillation counter. Counts per minute (cpm) were averaged for each triplicate dilution. The data was then plotted (cpm vs. log(concentration)) using Prism® by GraphPad Software and an IC_{50} was determined using that program's built-in one-site competition least squares regression function. K_i values were calculated by the equation: $K_i = IC_{50}/(1+ [^{3}\text{H-PDBu}])/K_d$ of ^{3}H-PDBu). The K_d of ^{3}H-PDBu was measured via saturation binding under identical conditions and was found to be 1.55 to 4.4 nM, depending on batch-to-batch variability.

Sample vial contents for PKC binding assay

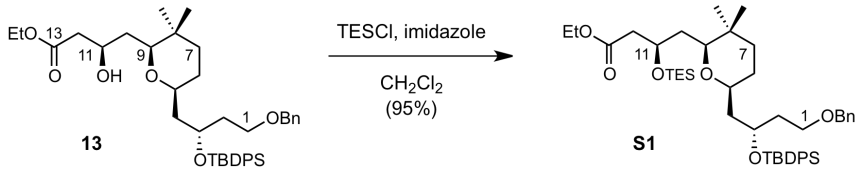
Constituent	*Stock concentration*	*Quantity*	*Final Concentration*
PS vesicle dilution	1 mg/mL	60 µL	66.6 µg/mL
PKC dilution	-	200 µL	-
^{3}H-PDBu	30 nM	20 µL	2 nM
Test Compound	Variable	20 µL	Variable
pH 7.4 Tris•HCl	50 mM	(from PKC and PS dilutions: 260 µL)	3.3 mM
KCl	100 mM		6.7 mM
$CaCl_2$	0.15 mM		10 µM
Bovine Serum Albumin	2 mg/mL		0.13 mg/mL

HIV Latency Induction Assay Protocol

J-Lat clone 10.6 cells were obtained from through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. These cells were cultured in RPMI Medium 1640 (Invitrogen) containing 10% fetal bovine serum (FBS, Omega Scientific) and 100 units/mL of Penicillin + 100 µg/mL of Streptomycin (Pen/Strep, Invitrogen). All cell culture procedures were performed in biosafety level 2+ conditions. During stimulation cells were seeded at a concentration of 25000 cells/well in a v-bottomed 96-well plate containing the relevant concentration of compound in 100 µl of RPMI media. Cells were incubated for 48 h and then harvested by washing with fresh media and resuspending pellets in 2% paraformaldehyde. The percentage of GFP+ cells was quantified by flow cytometry using fluorescence channel 1 on a FC 500 flow cytometer (Beckman Coulter). The resultant list mode files were processed using FlowJo software (version 7.6). EC_{50} values were calculated using nonlinear regression analysis in the GraphPad Prism software package (version 5).



Supporting Figure S1: Induction curves of GFP expression in the J-Lat cell line after dosing with Bryostatin 1, Prostratin, or analogues **1**-**7**. GFP expression indicates transcription of the HIV-Long Terminal Repeat and correlates with viral reactivation from latency.



Procedure for ester S1

To a solution of alcohol **13** (94.2 mg, 0.1425 mmol) in CH_2Cl_2 (1.4 mL) under ambient atmosphere was added imidazole (29.1 mg, 0.428 mmol). After the solids completely dissolved, chlorotriethylsilane (36 μL, 0.2145 mmol) was added in one portion, and a white suspension resulted. The mixture was stirred at room temperature for 15 minutes and was then diluted with saturated aqueous NH_4Cl (5 mL) and Et_2O (10 mL). The organic and aqueous layers were partitioned, and the aqueous layer was extracted with Et_2O (3 x 10 mL). The combined organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford a crude residue which was purified via silica gel column chromatography (0→4% EtOAc:Pentane) to yield desired ester **S1** (104.7 mg, 95%) as a clear, colorless oil.

Characterization data for **S1**:

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.62-7.72 (m, 4H), 7.22-7.45 (m, 11H), 4.37 (s, 2H), 4.19-4.30 (m, 1H), 3.96-4.16 (m, 3H), 3.52 (t, *J* = 6.9 Hz, 2H), 2.95-3.09 (m, 1H), 2.82 (d, *J* = 8.8 Hz, 1H), 2.40 (dd, *J* = 14.8, 4.14 Hz, 1H), 2.27 (dd, *J* = 14.8, 7.8 Hz, 1H), 1.64-1.90 (m, 3H), 1.55-1.63 (m, 1H), 1.43-1.53 (m, 2H), 1.19-1.37 (m, 6H), 1.03 (s, 9H), 0.92 (t, *J* = 7.9 Hz, 9H), 0.76 (s, 3H), 0.72 (s, 3H), 0.56 (q, *J* = 7.8 Hz, 6H) ppm.

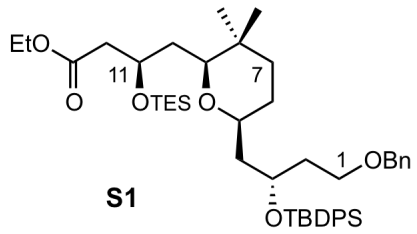
^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.5, 138.8, 136.1, 136.0, 134.8, 134.6, 129.6, 129.6, 128.4, 127.7, 127.7, 127.6, 127.5, 81.2, 75.4, 72.9, 69.4, 67.2, 67.1, 60.2, 44.8, 41.7, 39.4, 37.9, 37.7, 32.1, 28.7, 27.4, 27.2, 19.6, 19.1, 14.4, 7.0, 5.0 ppm.

IR (film): 2953, 2931, 2874, 2856, 1736, 1461, 1427, 1387, 1314, 1237, 1163, 1109, 1078, 821, 774, 737, 701, 611 cm^{-1}.

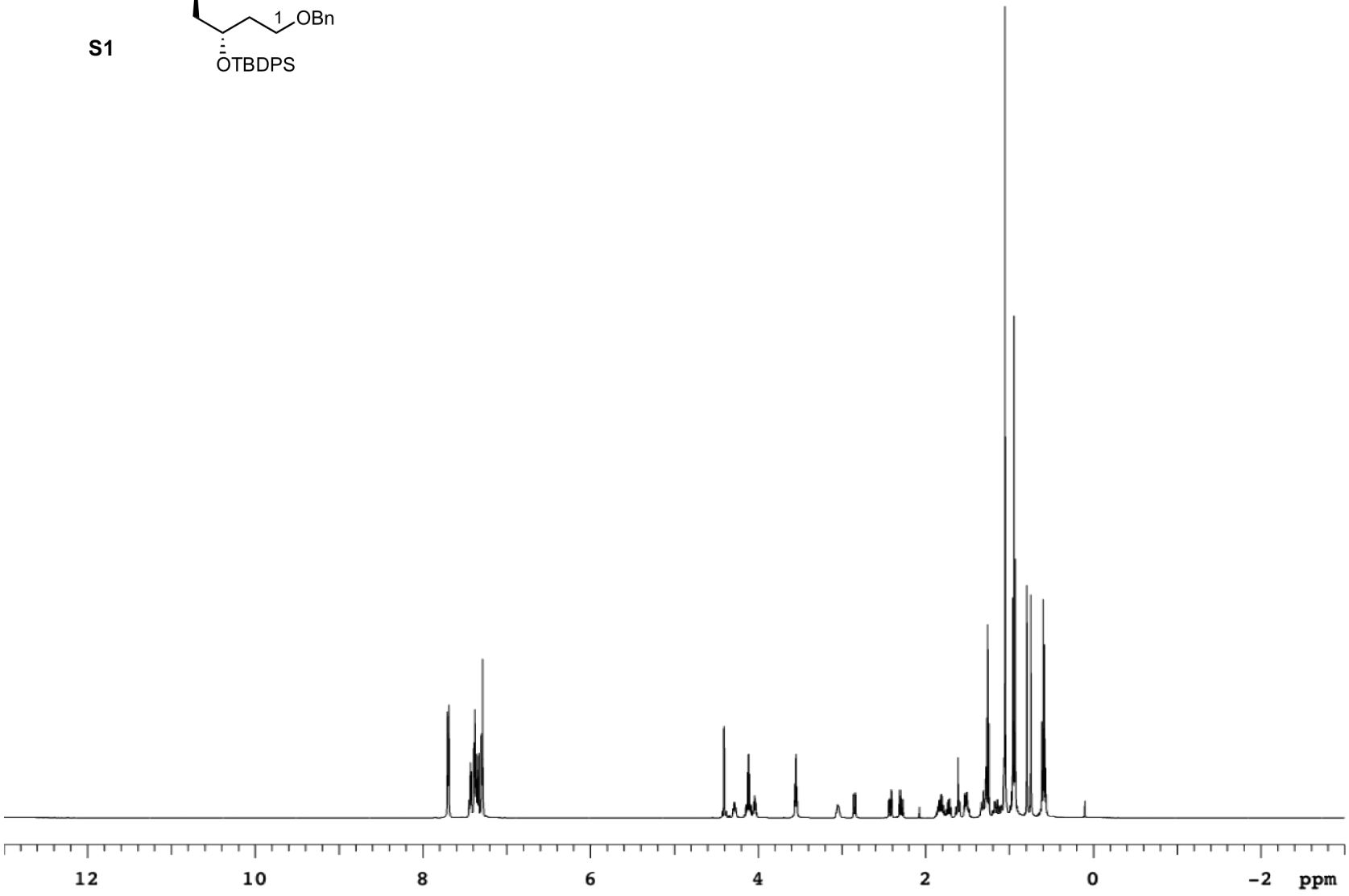
HRMS (TOF MS ES+): Calculated for $C_{46}H_{70}O_6Si_2Na^+$: 797.4609; Found: 797.4625.

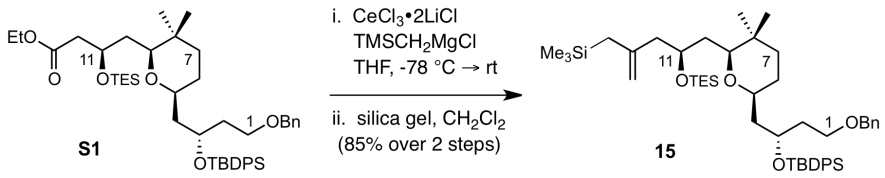
$[\alpha]_D^{25}$ = - 17.13° (*c* = 1.53, $CHCl_3$).

$\mathbf{R}_f$ = 0.75 (30% EtOAc, 70% Petroleum ether) – one brown spot (*p*-anisaldehyde stain).



S1





Procedure for allylsilane 15

To a N_2-purge 10 mL Schlenk flask was added a solution of $CeCl_3$•2LiCl (~0.31 M in THF, 1.48 mL, 0.46 mmol). The bronze solution was cooled in a CO_2/acetone bath for 5 min, and $TMSCH_2MgCl$ (0.97 M in Et_2O, 0.47 mL, 0.46 mmol) was added dropwise over 60 sec. The resulting tan suspension was stirred at -78 °C for 60 min, at which time ester **S1** (29.7 mg, 0.0383 mmol) was added via canula as a solution in THF (1.4 mL with 2 x 0.5 mL washes). The reaction mixture was then warmed to ambient temperature and stirred for 96 h. The reaction was quenched with saturated aqueous sodium potassium tartrate solution (5 mL) and the biphasic mixture was stirred vigorously for 15 min. The mixture was diluted with Et_2O (10 mL) and H_2O (10 mL), and the layers were separated. The aqueous layer was extracted with Et_2O (3 x 10 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated to afford a crude residue, which was used directly in the next step.

The crude product (max. 0.0383 mmol) from the previous step was dissolved in CH_2Cl_2 (1.6 mL). Silica gel (700 mg) was added in one portion, and the resulting white suspension was stirred at ambient temperature for 24 h. The suspension was then filtered through a plug of cotton, eluting with Et_2O and concentrated. The resulting crude residue was purified by silica gel chromatography (100% pentane → 3% → 5% EtOAc in pentane) to afford 26.6 mg allylsilane **15** (85% over 2 steps) as a clear, colorless oil.

Characterization data for **15**:

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.61-7.70 (m, 4H), 7.21-7.44 (m, 11H), 4.49 (d, *J* = 16.0 Hz, 2H), 4.36 (s, 2H), 3.88-4.03 (m, 2H), 3.52 (t, *J* = 6.9 Hz, 2H), 3.01 (q, *J* = 6.0, 12.0 Hz, 1H), 2.84 (dd, *J* = 10.2, 1.5 Hz, 1H), 2.12 (dd, *J* = 13.7, 3.8 Hz, 1H), 1.95 (dd, *J* = 14.3, 6.6 Hz, 1H), 1.66-1.88 (m, 4H), 1.60-1.65 (m, 1H), 1.46-1.55 (2H), 1.20-1.35 (m, 3H), 1.07-1.19 (m, 2H), 1.02 (s, 9H), 0.93 (t, *J* = 7.9 Hz, 9H), 0.74 (s, 3H), 0.70 (s, 3H), 0.56 (dq, *J* = 1.8, 7.6 Hz, 6H), 0.0 (9H) ppm.

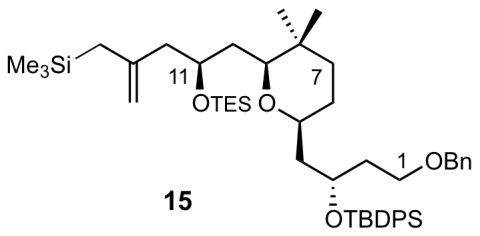
^{13}C NMR ($CDCl_3$, 125 MHz): δ 144.7, 138.8, 136.0, 136.0, 134.8, 134.5, 129.6, 129.6, 128.4, 127.7, 127.6, 127.6, 127.5, 110.2, 81.7, 75.4, 73.0, 69.4, 69.2, 67.2, 45.2, 44.9, 39.5, 38.3, 37.6, 32.3, 28.6, 27.5, 27.5, 27.2, 19.6, 19.1, 7.1, 5.1, -1.14 ppm.

IR (film): 3070, 2953, 2856, 1630, 1454, 1427, 1387, 1246, 1155, 1110, 1078, 1016, 851, 770, 737, 701, 611 cm^{-1}.

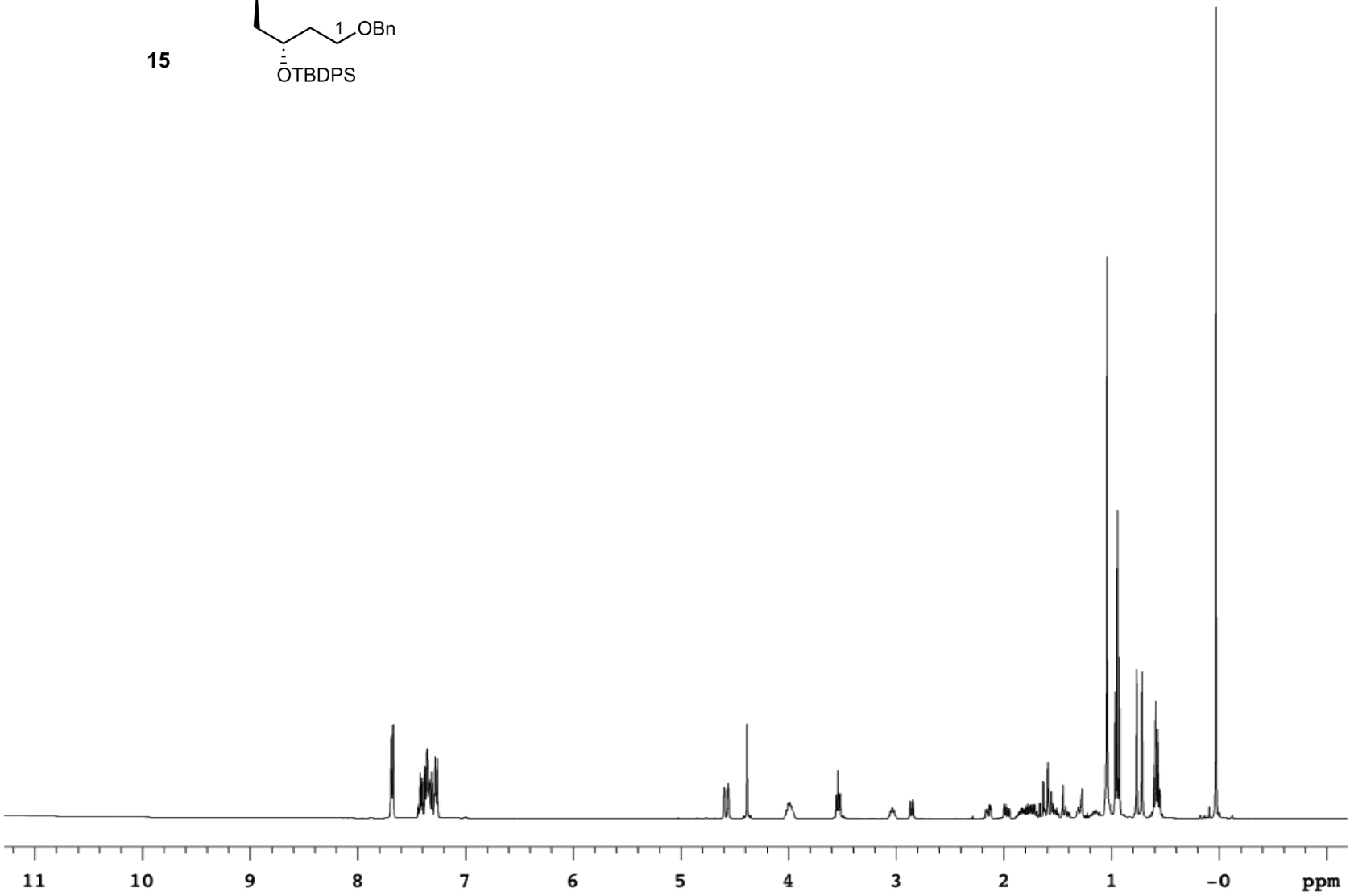
HRMS (TOF MS ES+): Calculated for $C_{49}H_{78}O_4Si_3Na^+$: 837.5106; Found: 837.5112.

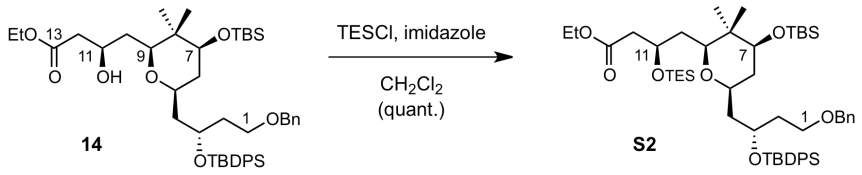
$[\alpha]_D^{25}$ = - 12.07° (*c* = 0.87, $CHCl_3$).

R_f = 0.7 (10% EtOAc, 90% Petroleum ether) – one purple spot (*p*-anisaldehyde stain).



15





Procedure for ester S2

Beta-hydroxyester **14** (48.8 mg, 0.062 mmol) was dissolved in CH_2Cl_2 (0.62 mL) in an oven-dried round bottom flask under N_2. Imidazole (12.6 mg, 0.185 mmol, Aldrich) was added in one portion, followed by addition of TESCl (15.5 mL, 0.093 mmol, Aldrich) dropwise via microsyringe over 3 s. The reaction was allowed to stir at room temperature for 21 h, at which point it was quenched with saturated NH_4Cl (3 mL). The reaction was diluted with H_2O (0.5 mL) and Et_2O (3 mL). The organic layer was extracted, and the aqueous layer was washed with Et_2O (3 x 4 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated *in vacuo*. Flash chromatography on silica (2.5% EtOAc: pentane) provided 57.6 mg (100%) of **S2** as a colorless oil.

Characterization data for **S2**:

^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.69-7.64 (4H, m, -OTBDPS); 7.44-7.24 (11H, m, -OTBDPS, -OCH_2Ph); 4.39 (2H, d, *J* = 2.7 Hz, -OCH_2Ph); 4.25-4.18 (1H, m, C11); 4.14-4.03 (2H, m, -C(O)OCH_2CH_3); 4.02-3.97 (1H, m, C3); 3.58-3.51 (2H, t, *J* = 7.1 Hz, C1); 3.08 (1H, dd, *J* = 4.8, 11.2 Hz, C7); 3.03-2.95 (1H, m, C5); 2.71 (1H, dd, *J* = 1.7, 9.7 Hz, C9); 2.37 (1H, dd, *J* = 4.2, 14.7 Hz, C12); 2.25 (1H, dd, *J* = 7.7, 14.7 Hz, C12); 1.89-1.73 (2H, m, C2); 1.73-1.65 (1H, m, C4); 1.65-1.50 (2H, m, C4, C10); 1.49-1.40 (1H, ddd, J = 14.1, 5.5, 4.7 Hz, C10); 1.23 (3H, t, J = 7.2 Hz, -C(O)OCH_2CH_3); 1.16-1.10 (1H, m, C6); 1.09-0.97 (1H, m, C6); 1.02 (9H, s, -OTBDPS); 0.91 (9H, t, *J* = 8.0 Hz, -OTES); 0.86 (9H, s, -OTBDMS); 0.73 (3H, s, -$C(CH_3)_2$); 0.68 (3H, s, -$C(CH_3)_2$); 0.56 (6H, q, *J* = 7.9 Hz, -OTES); -0.01 (3H, s, -OTBDMS); -0.05 (3H, s, -OTBDMS) ppm.

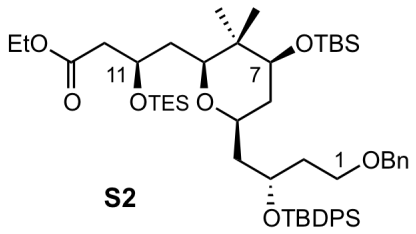
^{13}C-NMR (125 MHz, $CDCl_3$): δ 171.9, 138.8, 136.0 (2C), 134.8 (2C), 134.5 (2C), 129.73 (2C), 129.68 (2C), 128.4 (2C), 127.69 (2C), 127.66 (2C), 127.52, 79.8, 76.1, 72.96, 72.93 69.4, 67.3, 67.0, 60.2, 44.5, 41.6, 39.1, 37.76, 37.73, 37.3, 27.2 (3C), 26.0 (3C), 23.0, 19.6, 18.2, 14.4, 12.8, 7.0 (3C), 5.0 (3C), -3.7, -4.8 ppm.

IR (**film**): 2955, 2856, 1736, 1471, 1427, 1370, 1314, 1251, 1166, 1104, 862, 836, 773, 737, 702, 611 cm^{-1}.

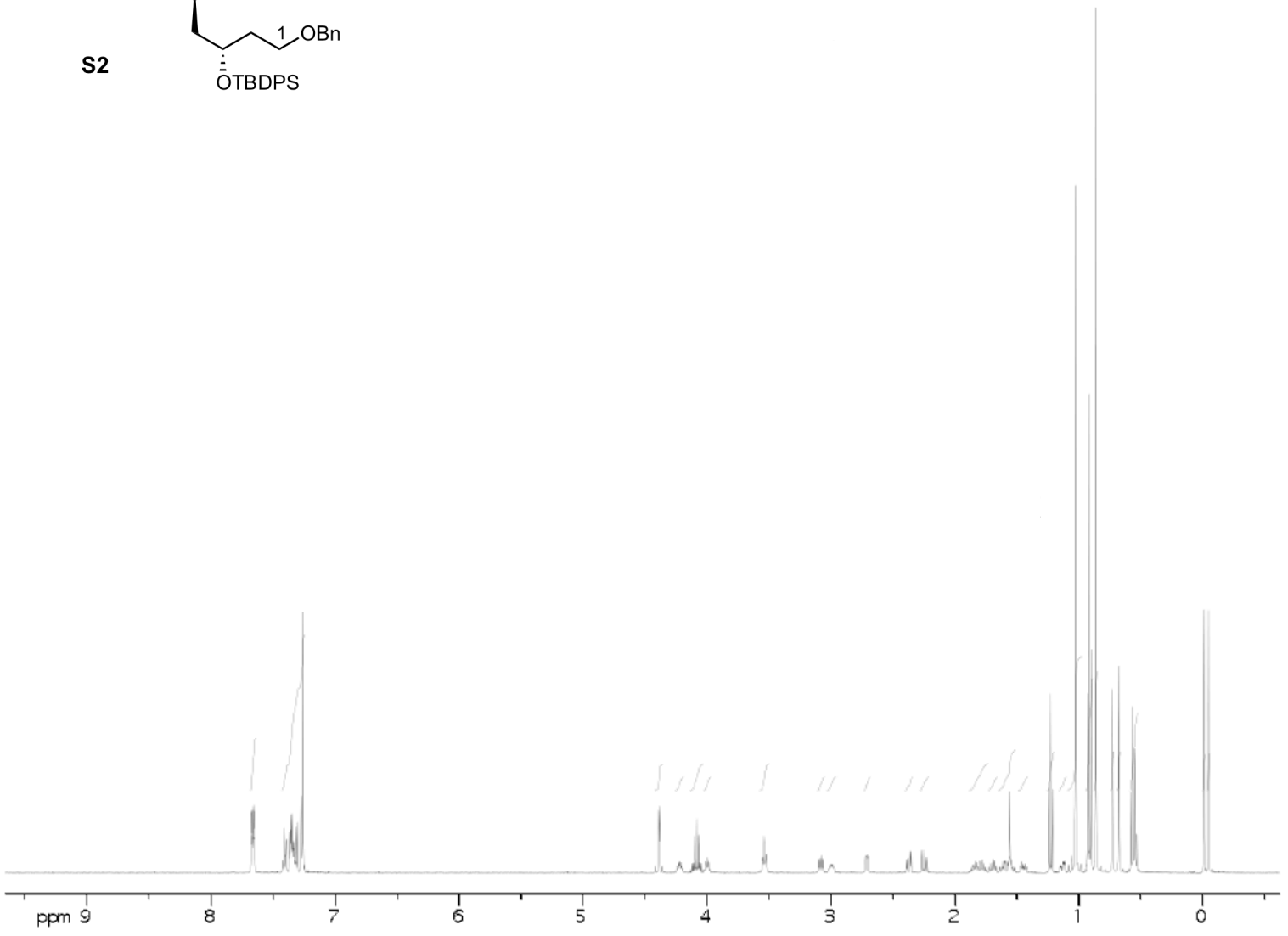
HRMS (TOF MS ES^+): calculated for $C_{52}H_{84}O_7Si_3Na$: 927.5423; Found: 927.5425.

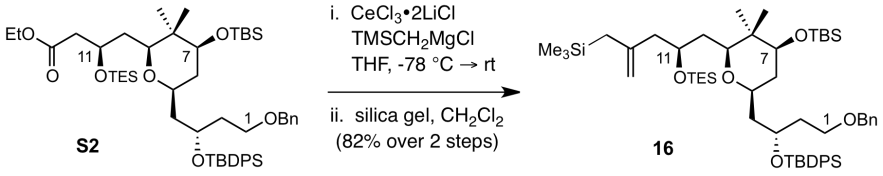
[a] $_D^{22.3}$ = -8.9 ^{o} (c = 0.19, CH_2Cl_2).

$\mathbf{R}_f$ = 0.41 (5% EtOAc/pentane) – one black spot, *p*-anisaldehyde stain.



S2





Procedure for allylsilane 16

To a N_2-purge 10 mL Schlenk flask was added a solution of $CeCl_3$•2LiCl (~0.28 M in THF, 9.0 mL, 2.52 mmol). The bronze solution was cooled in a CO_2/acetone bath for 5 min, and $TMSCH_2MgCl$ (0.94 M in Et_2O, 2.7 mL, 2.54 mmol) was added dropwise over 60 sec. The resulting tan suspension was stirred at -78 °C for 105 min, at which time ester **S2** (230 mg, 0.254 mmol) was added via canula as a solution in THF (2 mL) over 3 min. The reaction mixture was stirred at -78 °C for 20 min, then allowed to warm to ambient temperature and stirred for 42 h. The reaction was quenched with saturated aqueous sodium potassium tartrate solution (20 mL) and the biphasic mixture was stirred vigorously for 1 h. The mixture was diluted with Et_2O (20 mL), and the layers were separated. The aqueous layer was extracted with Et_2O (20 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated to afford a crude residue, which was used directly in the next step.

The crude product (max. 0.254 mmol) from the previous step was dissolved in CH_2Cl_2 (10 mL). Silica gel (2.0 g) was added in one portion, and the resulting white suspension was stirred at ambient temperature for 24 h. An additional 1.0 g silica gel was then added. After an additional 25 h, the suspension was then filtered through a plug of cotton, eluting with Et_2O and concentrated. The resulting crude residue was purified by silica gel chromatography (4% Et_2O in pentane) to afford 198 mg allylsilane **16** (82% over 2 steps) as a clear, colorless oil.

Characterization data for **16**:

^{1}H-NMR (500 MHz, C_6D_6): δ 7.83-7.78 (4H, m, -OTBDPS), 7.32-7.19 (10H, m, -OTBDPS, -OCH_2Ph), 7.14-7.08 (1H, m, -OCH_2Ph), 4.88 (1H, d, *J* = 2.2 Hz, C14), 4.77 (1H, d, *J* = 2.2 Hz, C14), 4.37 (1H, d, *J* = 12.1 Hz, -OCH_2Ph), 4.33 (1H, d, *J* = 12.1 Hz, -OCH_2Ph), 4.32-4.22 (2H, m, C3, C11), 3.74-3.68 (1H, m, C1), 3.61-3.55 (1H, m, C1), 3.22-3.14 (2H, m, C5, C7), 2.98-2.93 (1H, m, C9), 2.42 (1H, dd, *J* = 3.9, 13.8 Hz, C12), 2.23 (1H, dd, *J* = 6.4, 14.0 Hz, C12), 2.12-2.03 (1H, m, C2), 2.00-1.91 (2H, m, C2, C4), 1.89 (1H, dd, *J* = 0.5, 13.2 Hz, -CH_2TMS), 1.85-1.80 (2H, m, C10), 1.77 (1H, d, *J* = 13.3 Hz, -CH_2TMS), 1.66 (1H, dt, *J* = 4.9, 13.9 Hz, C4), 1.30-1.21 (2H, m, C6), 1.18 (9H, s, -OTBDPS), 1.09 (9H, t, *J* = 8.0 Hz, -OTES), 0.97 (9H, s, -OTBDMS), 0.95 (3H, s, -$C(CH_3)_2$), 0.88 (3H, s, -$C(CH_3)_2$), 0.73 (6H, q, *J* = 7.9 Hz, -OTES), 0.13 (9H, s, -TMS), 0.02 (3H, s, -OTBDMS), -0.01 (3H, s, -OTBDMS) ppm.

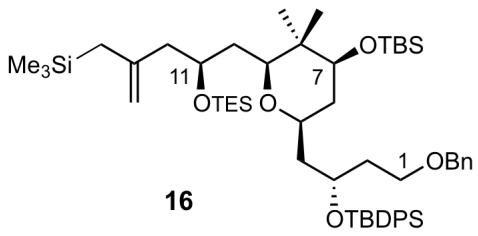
^{13}C-NMR (125 MHz, C_6D_6): δ 145.3, 139.4, 136.34, 136.33, 135.1 (2C), 134.7 (2C), 130.02 (2C), 129.99 (2C), 128.5 (2C), 128.03, 128.00, 127.83 (2C), 127.6, 110.6, 80.7, 76.7, 73.5, 73.2, 69.88, 69.83, 67.1, 45.5, 45.1, 39.5, 38.4, 38.16, 38.00, 27.8, 27.4 (3C), 26.1 (3C), 23.3, 19.8, 18.3, 13.1, 7.4 (3C), 5.6 (3C), -1.0 (3C), -3.7, -4.8 ppm.

IR (**film**): 2953, 2875, 2856, 1471, 1427, 1360, 1247, 1104, 1005, 858, 836, 773, 736, 700, 611 cm^{-1}.

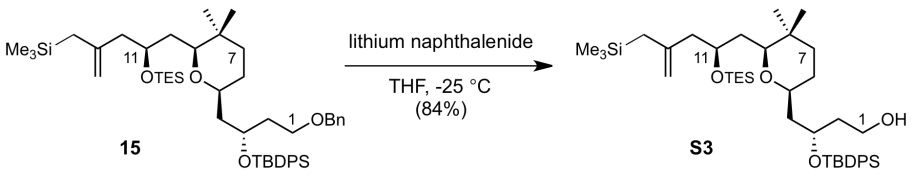
HRMS (TOF MS ES$^+$): calculated for $C_{55}H_{92}O_5Si_4Na$: 967.5920; Found: 967.5911.

$[\alpha]_D^{22.3}$ = -0.5 ^{o} (c = 2.0, CH_2Cl_2).

R_f = 0.55 (5% EtOAc/pentane) – one blue spot, *p*-anisaldehyde stain.



16



Procedure for alcohol S3

A solution of benzyl ether **15** (7.1 mg, 0.0087 mmol) in THF (0.15 mL) was cooled to -25 °C in a CO_2/MeCN bath. Lithium naphthalenide reagent (ca. 0.20 M in THF, 1.0 mL, 0.2 mmol) was added dropwise in 50 μL portions at 1-min intervals. After complete addition of the lithium naphthalenide reagent, the deep green mixture was allowed to stir for 10 minutes, after which TLC showed consumption of starting material. The reaction was quenched with saturated aqueous NH_4Cl (4 mL) and diluted with H_2O (4 mL) and Et_2O (10 mL). The layers were separated, and the aqueous layer was extracted with Et_2O (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting crude residue was purified via silica gel chromatography (5→8→15% EtOAc:Pentane) to provide alcohol **S3** (5.3 mg, 84%) as a clear, colorless oil.

Characterization data for **S3:**

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.63-7.75 (m, 4H), 7.33-7.48 (m, 6H), 4.54 (d, *J* = 12.6 Hz, 2H), 4.01 (dddd, *J* = 4.6, 4.6, 4.6, 9.0 Hz, 1H), 3.82-3.94 (m, 2H), 3.76 (ddt, *J* = 5.5, 5.5, 10.7 Hz, 1H), 2.81 (dd, *J* = 1.6, 10.0 Hz, 1H), 2.70-2.79 (m, 1H), 2.55-2.65 (m, 1H), 2.10 (dd, *J* = 4.0, 13.8 Hz, 1H), 1.90-2.01 (m, 2H), 1.86 (ddd, *J* = 7.7, 8.5, 14.0 Hz, 1H), 1.66-1.78 (m, 1H), 1.56 (s, 3H), 1.49-1.54 (m, 1H), 1.36-1.49 (m, 2H), 1.22-1.32 (m, 1H), 1.04-1.09 (m, 2H), 1.06 (s, 9H), 0.94 (t, *J* = 7.9 Hz, 9H), 0.74 (s, 3H), 0.69 (s, 3H), 0.56 (q, *J* = 8.1 Hz, 6H), 0.0 (s, 9H) ppm.

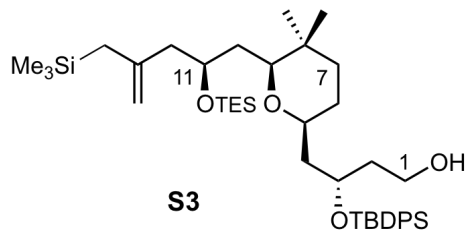
^{13}C NMR ($CDCl_3$, 125 MHz): δ 144.7, 136.0, 136.0, 134.2, 133.7, 130.0, 129.9, 127.9, 127.8, 110.1, 82.0, 76.0, 72.2, 69.1, 60.0, 45.3, 43.5, 39.4, 38.5, 38.5, 32.2, 28.8, 27.5, 27.4, 27.2, 19.4, 19.0, 7.1, 5.1, -1.2 ppm.

IR (film): 3467, 3071, 2953, 2876, 2858, 1630, 1472, 1461, 1427, 1387, 1363, 1321, 1246, 1155, 1110, 1073, 1007, 938, 851, 822, 770, 738, 701, 611 cm^{-1}.

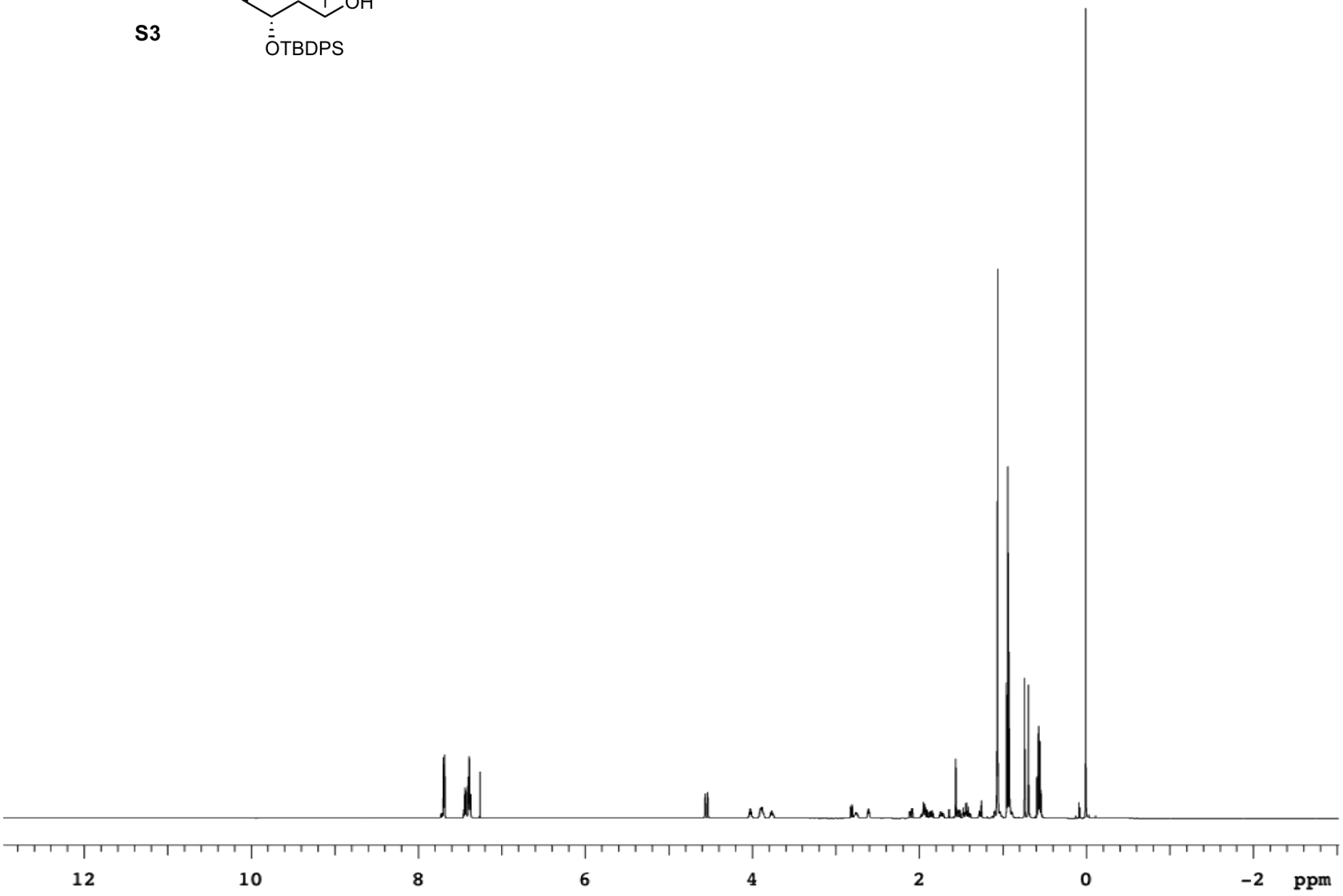
HRMS (TOF MS ES+): Calculated for $C_{42}H_{72}O_4Si_3Na^+$: 747.4636; Found: 747.4639.

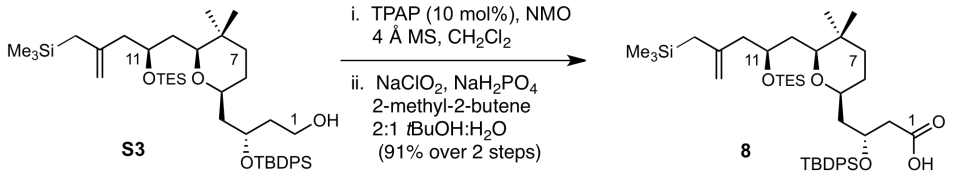
$[\alpha]_D^{25}$ = -20.00° (*c* = 0.985, $CHCl_3$).

$\mathbf{R}_f$ = 0.57 (10% EtOAc, 90% Petroleum ether) – one purple spot (*p*-anisaldehyde stain).



S3





Procedure for carboxylic acid northern fragment 8

To a small conical vial was added alcohol **S3** (5.3 mg, 0.0731 mmol) and powdered 4Å molecular sieves (27 mg). The vessel was purged with N_2. *N*-morpholine *N*-oxide (NMO) (2.6 mg, 0.022 mmol) was added as a solution in CH_2Cl_2 (125 μL). Tetrapropylammonium perruthenate (TPAP) (0.3 mg, 0.00085 mmol) was then added as a solution in CH_2Cl_2 (125 μL). The resulting green mixture was then allowed to stir at ambeint temperature for 2 h, at which time TLC showed consumption of starting material. The reaction material was loaded directly onto a short pad of silica gel and eluted with 20% EtOAc in pentane. The filtrate was concentrated to afford a clear oil that was used directly in the next step.

The oil from the last step (max. 0.0731 mmol) was dissolved in *t*BuOH (140 μL) at room temperature. H_2O (70 μL) was added, followed by 2-methyl-2-butene (55 μL). The resulting cloudy mixture was stirred vigorously and cooled in an ice water bath. To the suspension was added NaH_2PO_4 (8.8 mg, 0.073 mmol) followed by $NaClO_2$ (4.0 mg, 0.044 mmol). The mixture was stirred for 2 h and then quenched with 1:1 saturated aqueous $Na_2S_2O_3$:brine (2 mL). The resulting mixture was diluted with H_2O (6 mL) and Et_2O (8 mL). The layers were separated, and the aqueous layer was extracted with Et_2O (3 x 8 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting crude oil was purified via silica gel chromatography (10→15% EtOAc:Pentane) to yield carboxylic acid northern fragment **8** (4.9 mg, 91% over two steps) as a pale, yellow oil.

Characterization data for **8**:

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.63-7.75 (m, 4H, -SiPh), 7.33-7.49 (m, 6H, -SiPh), 4.55 (m, 1H, C14), 4.53 (m, 1H, C14), 4.12-4.21 (m, 1H, C3), 3.80-3.92 (m, 1H, C11), 2.79-2.96 (m, 2H, C5 & C9), 2.64 (dd, *J* = 4.4, 15.0 Hz, 1H, C2), 2.57 (dd, *J* = 5.5, 14.5 Hz, 1H, C2), 2.10 (dd, *J* = 4.8, 13.8 Hz, 1H, C12), 1.97 (dd, *J* = 6.5, 13.7 Hz, 1H, C12), 1.79 (dt, *J* = 8.8, 17.2 Hz, 1H, C4), 1.47-1.67 (m, 5H, C4, C10, C10, C30, C30), 1.35-1.46 (m, 2H, C7), 1.08-1.23 (m, 2H, C6), 1.04 (s, 9H, -Si^{t}Bu), 0.94 (t, *J* = 8.1 Hz, 9H, -Si($CH_2\underline{CH_3}$)$_3$), 0.76 (s, 3H, -C(CH_3)$_2$), 0.71 (s, 3H, -C(CH_3)$_2$), 0.57 (q, *J* = 8.1 Hz, 6H, -Si($\underline{CH_2}CH_3$)$_e$), 0.01 (s, 9H, -Si(CH_3)$_3$) ppm. (no COO$\underline{H}$ observed).

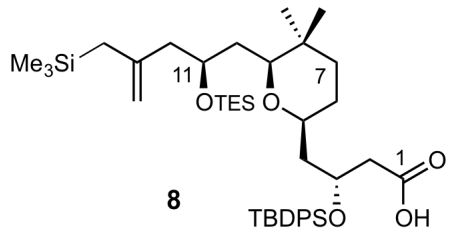
^{13}C NMR ($CDCl_3$, 125 MHz): δ 175.8, 144.5, 136.0, 136.0, 134.9, 134.2, 133.5, 129.9, 129.8, 127.8, 127.7, 110.3, 82.1, 75.5, 69.5, 69.3, 45.5, 44.1, 42.9, 39.4, 38.4, 32.3, 28.8, 27.5, 27.4, 27.0, 26.7, 19.4, 19.0, 7.1, 5.1, -1.2 ppm.

IR (film): 3071, 2954, 2858, 1712, 1632, 1462, 1427, 1388, 1310, 1247, 1191, 1155, 1111, 1084, 1006, 960, 851, 822, 738, 701, 611 cm^{-1}.

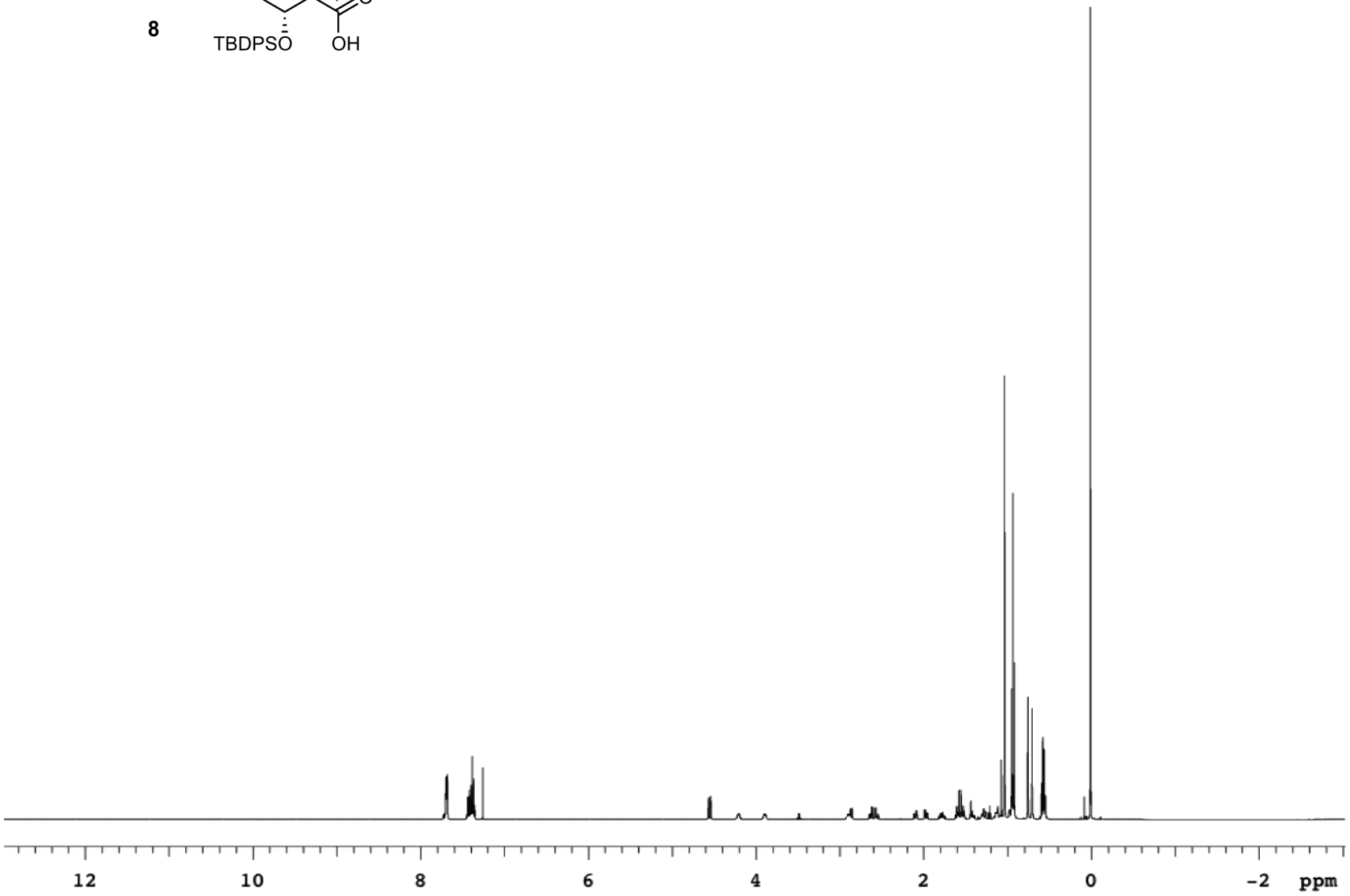
HRMS (TOF MS ES+): Calculated for $C_{42}H_{70}O_5Si_3Na^+$: 761.4429; Found: 761.4425.

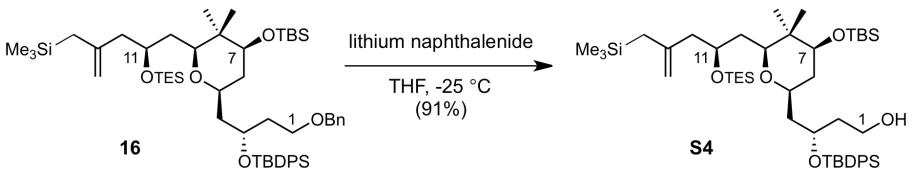
$[\alpha]_D^{24}$ = -11.94° (*c* = 1.04, $CHCl_3$).

R_f = 0.4 (20% EtOAc, 80% Pentroleum ether) – one purple spot (*p*-anisaldehyde stain).



8





Procedure for alcohol S4

Allylsilane **16** (17.8 mg, 0.203 mmol) was dissolved in THF (1.2 mL) in a flame-dried vial charged with a Teflon-coated magnetic stir bar under positive pressure of N_2 gas. The reaction was cooled to –20 ^oC (external temperature) in a NaCl/ice bath. A freshly prepared solution of lithium dissolved in naphthalene (1.2 mL, via two 30 min sonication cycles in a schlenk flask (ca 1.0 M in THF)) was then added dropwise via syringe over 6 min. After 40 min, the reaction was quenched with saturated NH_4Cl (5 mL) and allowed to warm to room temperature. The mixture was diluted with Et_2O (2 mL), and the organic layer was separated. The aqueous layer was extracted with Et_2O (5 x 1.5 mL), and the combined organic layers were dried with $MgSO_4$, filtered, and concentrated *in vacuo*. Flash chromatography on silica (2 → 5% EtOAc: Pentane) provided 156.8 mg (91%) of **S4** as a colorless oil.

Characterization data for **S4**:

^{1}H-NMR (500 MHz, C_6D_6): δ 7.83-7.76 (4H, m, -OTBDPS), 7.28-7.22 (6H, m, -OTBDPS), 4.85 (1H, d, *J* = 2.2 Hz, C14), 4.75 (1H, d, *J* = 2.2 Hz, C14), 4.27-4.17 (2H, m, C3, C11), 3.93-3.86 (1H, m, C1), 3.82-3.75 (1H, m, C1), 3.15 (1H, dd, *J* = 5.1, 11.0 Hz, C7), 3.01-2.94 (1H, m, C5), 2.94-2.90 (1H, m, C9), 2.38 (1H, dd, *J* = 3.7, 13.7 Hz, C12), 2.19 (1H, dd, *J* = 7.0, 13.8 Hz, C12), 2.08-1.99 (1H, m, C2), 1.97 (1H, dt, *J* = 7.8, 14.2 Hz, C4), 1.80 (2H, dd, *J* = 13.4, 45.4 Hz, -CH_2TMS), 1.89-1.76 (3H, m, C2, C10), 1.65 (1H, dd, *J* = 4.8, 5.8 Hz, C1OH), 1.59 (1H, dt, *J* = 4.3, 14.1 Hz, C4), 1.30-1.14 (2H, m, C6), 1.17 (9H, s, -OTBDPS), 1.09 (9H, t, *J* = 8.1 Hz, -OTES), 0.98 (9H, s, -OTBDMS), 0.94 (3H, s, -$C(CH_3)_2$), 0.87 (3H, s, -$C(CH_3)_2$), 0.73 (6H, q, *J* = 7.8 Hz, -OTES), 0.12 (9H, s, -TMS), 0.03 (3H, s, -OTBDMS), 0.00 (3H, s, -OTBDMS) ppm.

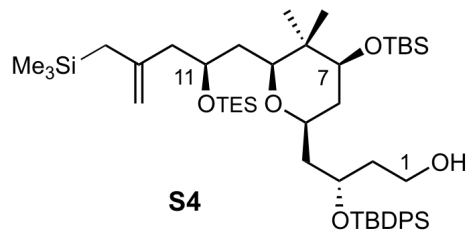
^{13}C-NMR (125 MHz, C_6D_6): δ 145.4, 136.32, 136.29, 134.8 (2C), 134.4 (2C), 130.13 (2C), 130.12 (2C), 128.13 (2C) 110.5, 80.8, 76.6, 73.8, 71.5, 69.7, 59.6, 45.5, 44.3, 40.2, 39.4, 38.3, 38.1, 27.7, 27.3 (3C), 26.1 (3C), 23.3, 19.6, 18.3, 13.0, 7.4 (3C), 5.6 (3C), -1.1 (3C), -3.7, -4.8 ppm.

IR (**film**): 3472, 3071, 2954, 2876, 2857, 1630, 1471, 1427, 1389, 1248, 1153, 1104, 1005, 858, 836, 773, 739, 701, 612 cm^{-1}.

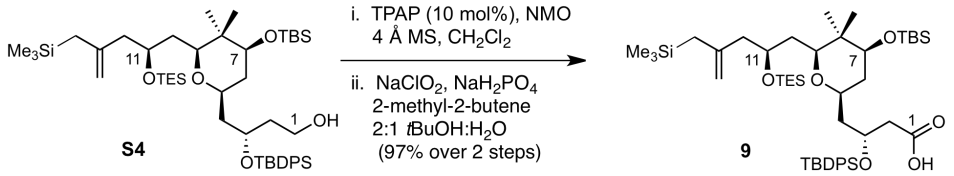
HRMS (TOF MS ES^+): calculated for $C_{48}H_{86}O_5Si_4Na$: 877.5450; Found: 877.5436.

$[\alpha]_D^{24.3}$ = - 10.6 ^{o} (c = 1.4, CH_2Cl_2).

R_f = 0.32 (5 % EtOAc/pentane) – one blue spot, *p*-anisaldehyde stain.



S4



Procedure for carboxylic acid northern fragment 9

Oven dried 4 Å molecular sieves were added to alcohol **S4** (32.2 mg, 0.038 mmol) in a round bottom flask charged with a Teflon coated magnetic stir bar and under positive pressure of N_2 gas. NMO (13.2 mg, 0.113 mmol, Aldrich) was then added as a solution in CH_2Cl_2 (0.63 mL) in one portion via syringe. Next, TPAP (1.3 mg, 0.004 mmol, Aldrich) was added as a solution in CH_2Cl_2 (0.63 mL) in one portion via syringe. The mixture was allowed to stir at room temperature for 1.5 h, at which point it was loaded onto a silica gel plug and eluted with 5% EtOAc: pentane (15 mL). The eluent was concentrated *in vacuo* to provide a crude, pale yellow oil.

This crude oil was dissolved in *t*-BuOH (1.2 mL) in a vial under ambient atmosphere charged with a Teflon coated magnetic stir bar. H_2O (0.6 mL) was added in one portion via syringe, followed by the addition of 2-methyl-2-butene (215 mL, 2.030 mmol Aldrich) in one portion via syringe. NaH_2PO_4 (44.7 mg, 0.372 mmol, Fisher) was then added in one portion, followed by addition of $NaClO_2$ (20.4 mg, 0.226 mmol, Aldrich) in one portion. The mixture was allowed to stir for 20 min, at which point it was quenched with 1:1 saturated NaS_2O_3:brine (2 mL) and diluted with H_2O (0.5 mL) and Et_2O (2 mL). The organic layer was separated, and the aqueous layer was washed with Et_2O (3 x 3 mL). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated. Flash chromatography on silica (7.5% EtOAc: Pentane) provided 31.6 mg (97%) **9** as a colorless oil.

Characterization data for **9**:

^{1}H-NMR (500 MHz, C_6D_6): δ 7.86-7.78 (4H, m, -OTBDPS), 7.30-7.22 (6H, m, -OTBDPS), 4.85 (1H, d, *J* = 1.9 Hz, C14), 4.77 (1H, d, *J* = 2.1 Hz, C14), 4.57-4.50 (1H, m, C3), 4.22-4.16 (1H, m, C11), 3.14 (1H, dd, *J* = 4.9, 11.3 Hz, C7), 3.03-2.96 (1H, m, C5), 2.92 (1H, dd, *J* = 3.1, 8.5 Hz, C9), 2.82 (1H, dd, *J* = 4.6, 15.2 Hz, C2), 2.75 (1H, dd, *J* = 7.3, 15.2 Hz, C2), 2.38 (1H, dd, *J* = 4.2, 13.6 Hz, C12), 2.22 (1H, dd, *J* = 6.6, 13.8 Hz, C12), 1.94-1.73 (5H, m, C4, C10, -CH_2TMS), 1.60 (1H, dt, *J* = 3.8, 14.2 Hz, C4), 1.33-1.16 (2H, m, C6), 1.20 (9H, s, -OTBDPS), 1.12 (9H, t, *J* = 8.0 Hz, -OTES), 0.97 (9H, s, -OTBDMS), 0.93 (3H, s, -$C(CH_3)_2$), 0.86 (3H, s, -$C(CH_3)_2$), 0.76 (6H, q, *J* = 7.8 Hz, -OTES), 0.14 (9H, s, -TMS), 0.03 (3H, s, -OTBDMS), -0.01 (3H, s, -OTBDMS) ppm.

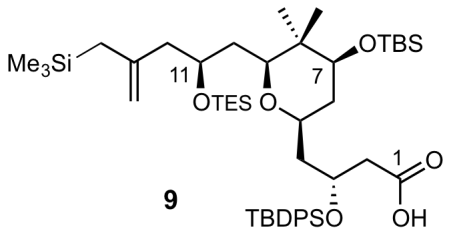
^{13}C-NMR (125 MHz, C_6D_6): δ 176.9, 145.2, 136.38, 136.28, 134.7 (2C), 134.1 (2C), 130.19 (2C), 130.11 (2C), 128.12 (2C), 110.7, 80.8, 76.5, 73.4, 70.2, 69.8, 45.7, 44.5, 43.4, 39.5, 38.26, 38.13, 27.8, 27.3 (3C), 26.1 (3C), 23.3, 19.7, 18.3, 13.0, 7.4 (3C), 5.6 (3C), -1.0 (3C), -3.7, -4.8 ppm

IR (**film**): 2955, 2932, 2857, 1712, 1427, 1248, 1110, 1083, 1005, 858, 836, 773, 738, 701 cm^{-1}.

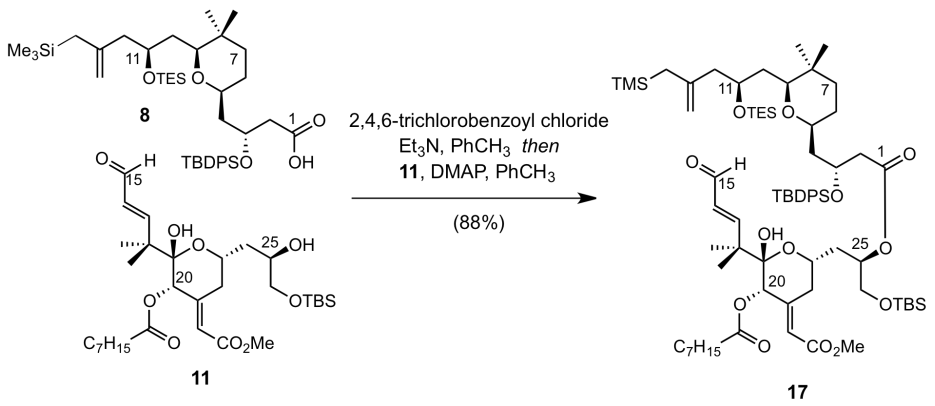
HRMS (TOF MS ES^+): Calculated for $C_{48}H_{84}O_6Si_4Na$: 891.5243; Found: 891.5242.

$[\alpha]_D^{22.9}$ = - 4.2 ^{o} (c = 1.1, CH_2Cl_2).

R_f = 0.38 (7.5% EtOAc: pentane) – one purple spot, *p*-anisaldehyde stain.



9



Procedure for macrocyclization precursor 17

Carboxylic acid **8** (4.9 mg, 0.0066 mmol) was dissolved in toluene (200 μL) in a small oven-dried vial equipped with a Teflon-coated stirbar under N_2. To this solution was added triethylamine (4.3 μL, 0.031 mmol), then 2,4,6-trichlorobenzoyl chloride (1.2 μL, 0.0077 mmol). The resulting solution was stirred for 4 h at ambeint temperature, and the formation of salts was observed. A toluene solution of recognition domain **11** (4.8 mg, 0.0082 mmol) and DMAP (2.0 mg, 0.016 mmol) was then added in toluene (100 μL) via cannula (with 2 x 75 μL and 1 x 50 μL washes). The resulting solution was stirred for 45 min, at which time it was loaded directly onto a slurry-packed silica gel column. Purification by column chromatography (5→7.5→10→15% EtOAc:Pentane) provided desired macrocyclization precursor **17** (7.7 mg, 88%) as a pale yellow oil.

Characterization data for **17**:

^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.56 (d, *J* = 7.8 Hz, 1H, C15), 7.61-7.72 (m, 4H, -SiPh), 7.31-7.46 (m, 7H, -SiPh & C17), 6.00 (d, *J* = 1.83 Hz, 1H, C27), 5.96, (dd, *J* = 7.8, 16.1 Hz, 1H, C16), 5.19 (m, 1H, C25), 5.12 (s, 1H, C20), 4.55 (bs, 1H, C14), 4.53 (bs, 1H, C14), 4.11-4.26 (m, 1H, C3), 3.86-3.98 (m, 1H, C11), 3.81 (bt, *J* = 11.6 Hz, 1H, C23), 3.70 (dd, *J* = 5.0, 10.7 Hz, 1H, C22), 3.61-3.68 (m, 2H, C26), 3.65 (s, 3H, -CO_2Me), 3.13 (s, 1H, C19-OH), 2.89-3.00 (m, 1H, C5), 2.84 (dd, *J* = 1.3, 9.8 Hz, 1H, C9), 2.51 (dd, *J* = 7.1, 15.3 Hz, 1H, C2), 2.46 (dd, *J* = 4.8, 15.3 Hz, 1H, C2), 1.88-2.17 (m, 6H, C12 & C12 & C22 & C24 & C31 & C31), 1.65-1.80 (m, 2H, C4 & C24), 1.52-1.64 (m, 3H, C10 & C30 & C30), 1.44-1.52 (m, 3H, C4 & C32 & C32), 1.34-1.42 (ddd, *J* = 3.3, 10.0, 13.6 Hz, 1H, C10), 1.08-1.32 (m, 12H, C6 & C7 & C33 & C34 & C35 & C36), 1.17 (s, 3H, C28), 1.16 (s, 3H, C29), 0.98 (s, 9H, -Si^{t}Bu), 0.92 (t, *J* = 8.2 Hz, 9H, -Si($CH_2\underline{CH_3}$)$_3$), 0.91 (s, 9H, -Si^{t}Bu), 0.87 (t, *J* = 7.0 Hz, 3H, C37), 0.73 (s, 3H, C(8)$(CH_3)_2$), 0.69 (s, 3H, C(8)$(CH_3)_2$), 0.56 (q, *J* = 8.2 Hz, 6H, -Si($\underline{CH_2}CH_3$)$_e$), 0.08 (s, 3H, -SiMe), 0.07 (s, 3H, -SiMe), 0.01 (s, 9H, -Si$(CH_3)_3$) ppm.

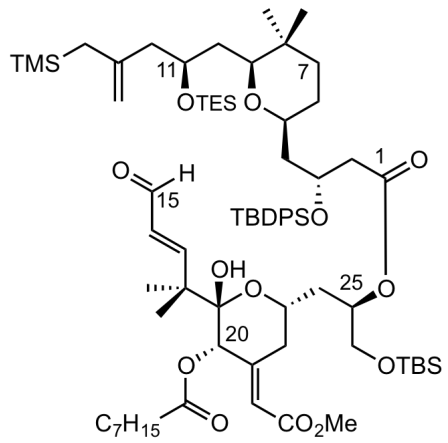
^{13}C NMR ($CDCl_3$, 125 MHz): δ 194.8, 172.8, 171.9, 166.8, 166.5, 150.6, 144.7, 136.0, 136.0, 134.3, 133.8, 129.8, 129.7, 127.7, 127.7, 127.4, 120.9, 110.1, 99.7, 81.8, 77.4, 75.0, 72.6, 71.8, 69.3, 68.6, 66.3, 65.1, 51.3, 45.9, 45.3, 44.3, 42.7, 39.4, 38.4, 37.8, 34.7, 32.3, 31.7, 31.0, 29.0, 29.0, 28.4, 27.5, 27.5, 27.1, 26.0, 24.6, 23.2, 22.7, 20.1, 19.4, 19.0, 18.4, 14.2, 7.2, 5.1, -1.1, -5.1 ppm.

IR (film): 3479, 2953, 2928, 2855, 1724, 1691, 1463, 1428, 1387, 1248, 1155, 1110, 838, 776, 739, 702 cm^{-1}.

HRMS (TOF MS ES+): Calculated for $C_{73}H_{122}O_{13}Si_4Na^+$: 1341.7860; Found: 1341.7866.

$[\alpha]_D^{25}$ = -34.64° (*c* = 0.90, $CHCl_3$).

$\mathbf{R}_f$ = 0.63 (20% Et_2O, 80% Petroleum ether) – one purple spot (*p*-anisaldehyde stain).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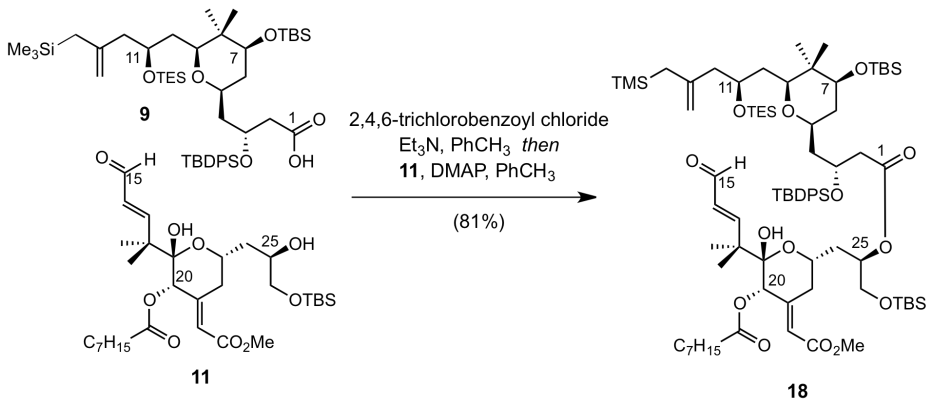
7
OTES
O
1
O
O
H
15
TBDPSO
OH
O
25
O
20
OTBS
O
C_7H_{15}
O
CO_2Me

17

12
10
8
6
4
2
0
-2
ppm



Procedure for macrocyclization precursor 18

Acid **9** (50.3 mg, 0.0578 mmol) was dissolved in toluene (1.9 mL) in a glass vial charged with a Teflon-coated magnetic stir bar and under positive pressure of N_2 gas. Et_3N (50 μL, 0.359 mmol) was added in one portion via microsyringe, followed by addition of 2,4,6-trichlorobenzoyl chloride (14.5 μL, 0.0886 mmol) in one portion via microsyringe. The reaction was allowed to stir for 4 h at room temperature, at which point recognition domain **11** (34.2 mg, 0.0586 mmol) was added as a solution of toluene (0.9 mL with 2 x 0.5 mL washes) and DMAP (20.9 mg, 0.174 mmol) dropwise via canula. The reaction was allowed to stir at room temperature for 35 min, at which point it was loaded directly onto a silica gel column and eluted with 6-10% EtOAc: pentane to provide 68 mg (81%) of **18** as a colorless oil.

Characterization data for **18**:

^{1}H-NMR (500 MHz, C_6D_6): δ 9.73 (1H, d, *J* = 7.6 Hz, C15); 7.86-7.81 (4H, m, -OTBDPS), 7.49 (1H, d, *J* = 16.1 Hz, C17), 7.36-7.22 (6H, m, -OTBDPS), 6.34 (1H, d, *J* = 1.8 Hz, C27), 6.05 (1H, dd, *J* = 7.6, 16.1 Hz, C16), 5.53-5.47 (1H, m, C25), 5.51 (1H, s, C20), 4.86 (1H, s, C14), 4.82 (1H, s, C14), 4.53-4.46 (1H, m, C3), 4.20-4.14 (3H, m, C11, C22, C23), 3.70 (1H, dd, *J* = 5.4, 10.6 Hz, C26), 3.64 (1H, dd, *J* = 5.7, 10.7 Hz, C26), 3.29 (3H, s, -OMe), 3.28 (1H, bs, C19OH), 3.19-3.12 (1H, m, C7), 3.06-2.98 (1H, m, C5), 2.92 (1H, dd, *J* = 1.8, 9.3 Hz, C9), 2.73 (1H, dd, *J* = 7.4, 15.5 Hz, C2), 2.62 (1H, dd, *J* = 4.3, 15.4 Hz, C2), 2.42-2.30 (2H, m, C12, C22), 2.20 (1H, dd, *J* = 6.8, 13.8 Hz, C12), 2.06-1.93 (4H, m, C4, C24, C31), 1.90-1.69 (5H, m, C10, C24, -CH_2TMS), 1.66 (1H, dt, *J* = 4.6, 14.2 Hz, C4), 1.55-1.41 (2H, m, C32), 1.30-1.08 (8H, m, C6, C33, C35, C36), 1.24 (3H, s, -C(CH_3)$_2$), 1.20 (9H, s, -OTBDPS), 1.19 (3H, s, -C(CH_3)$_2$), 1.13 (9H, t, *J* = 8.1 Hz, -OTES), 1.00 (9H, s, -OTBDMS), 0.99 (9H, s, -OTBDMS), 1.01-0.96 (2H, m, C34), 0.94 (3H, s, -C(CH_3)$_2$), 0.89 (3H, t, *J* = 7.2 Hz, C37), 0.89 (3H, s, -C(CH_3)$_2$), 0.76 (6H, q, *J* = 7.8 Hz, -OTES), 0.17 (9H, s, TMS), 0.10 (3H, s, -OTBDMS), 0.10 (3H, s, -OTBDMS), 0.06 (3H, s, -OTBDMS), 0.04 (3H, s, -OTBDMS) ppm.

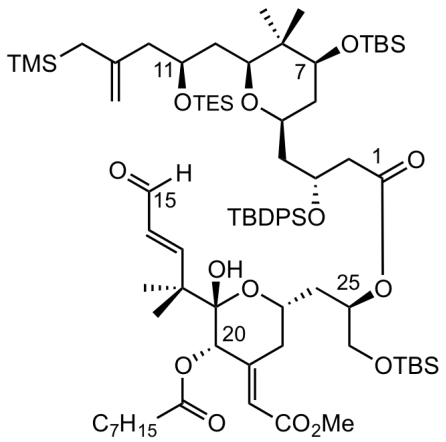
^{13}C-NMR (125 MHz, C_6D_6): δ 190.6, 173.0, 171.4, 166.2, 164.9, 151.0, 145.4, 136.34, 136.28, 134.6 (2C), 134.0 (2C), 130.27 (2C), 130.18 (2C), 121.4, 110.5, 100.2, 80.7, 76.5, 73.08, 72.93, 72.2, 70.4, 69.4, 66.7, 65.4, 50.8, 45.9, 45.5, 44.3, 43.1, 39.5, 38.20, 38.07, 34.7, 32.0, 31.5, 30.29, 30.24, 29.29, 29.27, 27.9, 27.3 (3C), 26.11 (3C), 26.10 (3C), 24.9, 23.3, 23.13, 23.00, 20.3, 19.7, 18.6, 18.3, 14.3, 13.0, 7.5 (3C), 5.6 (3C), -1.0 (3C), -2.5, -3.7, -4.7, -5.1 (2 TBDPS carbons obstructed by C_6D_6 signal) ppm.

IR (thin film): 2954, 2856, 1724, 1690, 1471, 1428, 1388, 1361, 1249, 1154, 1110, 1006, 983, 837, 774, 739, 701 cm^{-1}.

HRMS (TOF MS ES^+): Calculated for $C_{79}H_{136}O_{14}Si_5Na$: 1471.8674; Found: 1471.8680.

$[\alpha]_D^{23.1}$ = -20.7 ^{o} (c = 1.1, CH_2Cl_2).

$\mathbf{R}_f$ = 0.25 (7% EtOAc: pentane) – one black spot, *p*-anisaldehyde stain.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OTBS
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H
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TBDPSO
OH
O
25
O
20
O
OTBS
O
C_7H_{15}
O
CO_2Me

18

10
9
8
7
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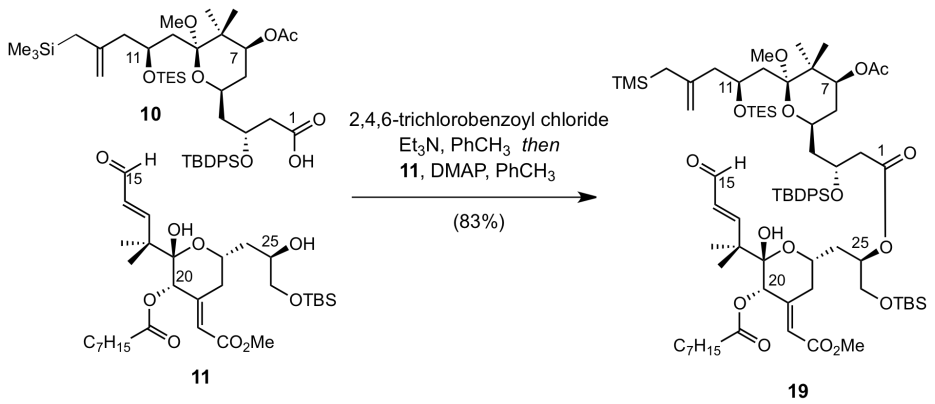
5
4
3
2
1
0



Procedure for macrocyclization precursor 19

Carboxylic acid **10** (18.9 mg, 0.0229 mmol) was dissolved in $PhCH_3$ (1.0 mL). Et_3N (19 µL, 0.14 mmol) was added in one portion followed by 2,4,6-trichlorobenzoyl chloride (5.7 µL, 0.034 mmol). The resulting solution was stirred at ambient temperature for 4 h, during which time formation of salts was observed. Recognition domain **11** (14.4 g, 0.0240 mmol) and DMAP (9.2 mg, 0.075 mmol) were then added by canula as a solution in $PhCH_3$ (500 µL); additional portions of $PhCH_3$ (2 x 250 µL) were added in the same manner to ensure complete transfer. The resulting mixture was stirred for 40 min at ambient temperature and was then loaded directly onto a slurry-packed silica gel column. Elution with 7 → 14 % EtOAc in hexanes provided 26.6 mg of ester **19** (83%) as a white foam. Compound purity was established by TLC (single spot) and 1H-NMR analysis.

Characterization data for **19**:

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 9.56 (d, 1H, *J* = 7.8 Hz, C15), 7.65 (m, 4H, -TBDPS), 7.44-7.32 (m, 6H, -TBDPS), 5.99 (d, 1H, *J* = 1.7 Hz, C34), 5.95 (dd, 1H, *J* = 7.8, 16.1 Hz, C16), 5.21 (m, 1H, C25), 5.12 (s, 1H, C20), 4.93 (dd, 1H, *J* = 4.8, 11.7 Hz, C7), 4.61 (bs, 1H, C14), 4.56 (bs, 1H, C14), 4.11 (m, 2H, C3, C11), 3.82 (tt, 1H, *J* = 1.8, 11.4 Hz, C23), 3.69 (dd, 1H, *J* = 5.1, 10.8 Hz, C26), 3.68-3.60 (m, 2H, C22, C26), 3.64 (s, 3H, -CO_2Me), 3.31 (m, 1H, C5), 3.12 (s, 1H, C19-OH), 2.94 (s, 3H, C9-OMe), 2.52 (m, 2H, C2, C2), 2.13-1.91 (m, 6H, C12, C12, C22, C24, C40, C40), 2.01 (s, 3H, C7-OAc), 1.86- 1.67 (m, 4H, C4, C10, C10, C24), 1.53-1.44 (m, 5H, C4, C30, C30, C41, C41), 1.29-1.18 (m, 9H, C6, C42-C45), 1.17 (s, 3H, -Me), 1.15 (s, 3H, -Me), 0.98 (s, 9H, -TBDPS), 0.93 (t, 9H, *J* = 7.9 Hz, -TES), 0.91 (s, 9H, -TBS), 0.87 (s, 3H, -Me), 0.87 (t, 3H, *J* = 6.9 Hz, C46), 0.86 (s, 3H, -Me), 0.82 (m, 1H, C6), 0.56 (q, 6H, *J* = 7.9 Hz, -TES), 0.07 (s, 3H, -TBS), 0.06 (s, 3H, -TBS), 0.03 (s, 9H, -TMS) ppm.

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 194.8, 172.3, 171.8, 170.8, 166.8, 166.5, 150.4, 144.3, 136.0, 135.9, 134.0, 133.5, 129.9, 129.8, 127.7, 127.7, 127.5, 121.0, 110.7, 104.2, 99.6, 74.1, 72.7, 71.6, 68.3, 68.1, 66.3, 65.1, 65.0, 51.3, 48.6, 48.3, 45.8, 43.4, 42.4, 41.9, 39.7, 37.7, 34.6, 32.4, 31.7, 31.0, 29.0[3], 27.4, 27.0, 26.0, 24.6, 23.1, 22.7, 21.4, 20.8, 20.1, 19.4, 18.4, 17.1, 14.2, 7.2, 5.6, -1.3, -5.1[3] ppm.

IR: 3486, 3072, 2953, 2858, 2722, 1738, 1731, 1715, 1693, 1631, 1471, 1463, 1428, 1386, 1367, 1249,

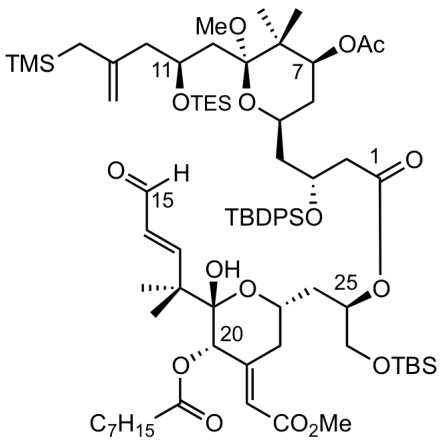
[3] Two resonances fail to resolve at this chemical shift. Verified by HMBC/HSQC.

1154, 1111, 1035, 839, 777, 739, 703 cm^{-1}

HRMS (TOF MS ES+): Calculated for: $C_{76}H_{126}O_{16}Si^{4}Na^{+}$: 1429.8015; Found: 1429.8066.

$[\alpha]_D^{25} = +1.9 \pm 0.3\ ^\circ$ (*c* = 1.07, CH_2Cl_2)

$\mathbf{R}_f$ = 0.50 (20% EtOAc in petroleum ether), one blue spot, *p*-anisaldehyde stain.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11
7
OTES
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1
O
H
O
15
TBDPSO
OH
O
25
O
20
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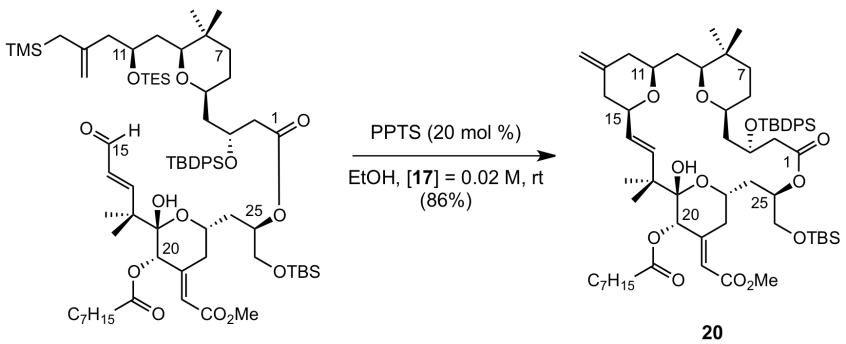
O
C_7H_{15}
O
CO_2Me

19

10
9
8
7
6
5
4
3
2
1
ppm



Procedure for bryopyran 20

Neat macrocyclization precursor **17** (10.3 mg, 0.0078 mmol) was dissolved in a solution of pyridinium *para*-toluenesulfonate (PPTS) in anhydrous EtOH (4 mM, 0.39 mL, 0.0016 mmol). The reaction was stirred under ambient atmosphere in a Teflon-capped vial for 4 h, at which time the solution was diluted with 30% Et_2O in petroleum ether (2 mL), H_2O (0.5 mL) and brine (1.5 mL). The phases were separated, and the aqueous phase was extracted with 30% Et_2O in petroleum ether (3 x 2 mL). The combined organic phase was dried over $MgSO_4$, filtered, and concentrated to afford a crude residue that was purified via silica gel chromatography (5→8→11→15% EtOAc:Pentane) to afford 7.5 mg bryopyran **20** (86%) as a clear, colorless oil.

Characterization data for **20**:

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.63-7.79 (m, 4H, -SiPh), 7.31-7.46 (m, 6H, -SiPh), 6.00 (d, J = 1.7 Hz, 1H, C27), 5.94 (d, J = 15.9 Hz, 1H, C17), 5.55, (dd, J = 8.7, 15.9 Hz, 1H, C16), 5.23 (m, 1H, C25), 5.14 (s, 1H, C20), 4.84 (bs, 1H, C30), 4.83 (bs, 1H, C30), 4.49 (m, 1H, C3), 3.98 (bt, J = 11.2 Hz, 1H, C23), 3.80-3.91 (m, 2H, C15 & C26), 3.68 (s, 3H, , -CO_2Me), 3.61-3.75 (m, 1H, C22), 3.43-3.52 (m, 1H, C26), 3.20 (m, 1H, C11), 2.90 (s, 1H0, C19-OH), 2.67 (dd, J = 2.0, 15.1 Hz, 1H, C2), 2.51 (dd, J = 10.5, 15.1 Hz, 1H, C2), 2.26-2.41 (m, 4H, C5 & C9 & C31 & C31), 2.15-2.26 (m, 2H, C12 & C14), 1.95-2.13 (m, 3H, C14 & C22 & C24), 1.69-1.91 (m, 2H, C12 & C24), 1.62 (m, 2H, C32), 1.13-1.45 (m, 16H, C4 & C6 & C7 & C10 & C33-36), 1.24 (s, 3H, C28), 1.04 (s, 3H, C29), 0.98 (s, 9H, -Si^{t}Bu), 0.91 (s, 9H, -Si^{t}Bu), 0.88 (t, J = 7.2 Hz, 3H, C37), 0.66 (s, 3H, C(8)$(CH_3)_2$), 0.63 (s, 3H, C(8)$(CH_3)_2$), 0.08 (s, 3H, -SiMe), 0.05 (s, 3H, -SiMe) ppm.

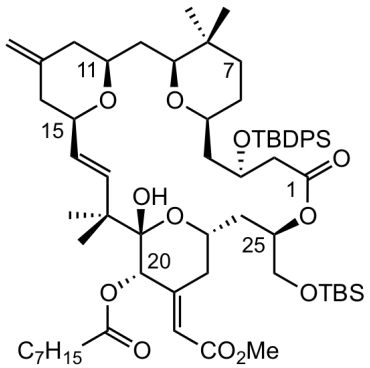
^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.3, 172.1, 166.8, 151.6, 143.9, 138.6, 136.2, 135.9, 135.1, 133.8, 132.9, 129.7, 129.5, 127.8, 127.6, 119.7, 109.2, 83.9, 82.4, 80.5, 76.5, 76.1, 74.3, 71.0, 69.7, 65.4, 64.5, 52.3, 45.3, 44.3, 41.2, 40.6, 38.8, 38.5, 37.3, 34.8, 31.8, 31.8, 31.5, 29.8, 29.2, 29.0, 28.6, 27.2, 27.0, 26.0, 24.8, 24.1, 22.7, 19.7, 19.5, 18.7, 18.4, 14.2, -5.1, -5.1 ppm.

IR (film): 3417, 2927, 2852, 1736, 1666, 1471, 1428, 1385, 1362, 1250, 1229, 1158, 1104, 837, 777, 703 cm^{-1}.

HRMS (TOF MS ES+): Calculated for $C_{64}H_{98}O_{12}Si_2Na^+$: 1137.6495; Found: 1137.6505.

$[\alpha]_D^{25}$ = -14.96° (c = 0.82, $CHCl_3$).

R_f = 0.55 (20% EtOAc, 80% Pentane) – one purple spot (*p*-anisaldehyde stain).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O
1
OH
O
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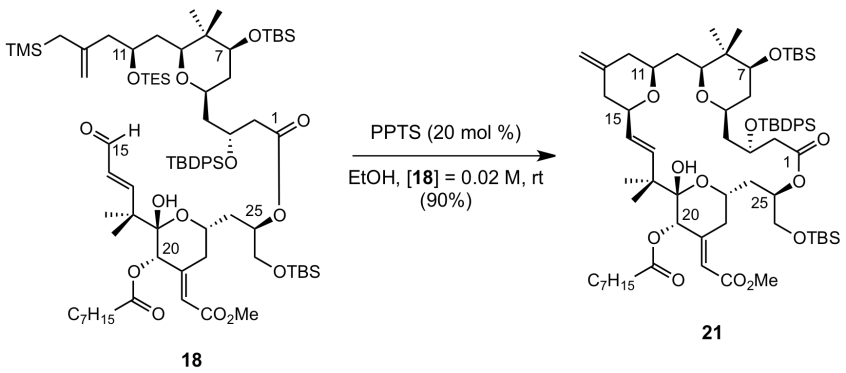
25
20
OTBS
O
C7H15
O
CO2Me

20

12
10
8
6
4
2
0
-2
ppm



Procedure for bryopyran 21

Neat ester **18** (48 mg, 0.033 mmol) was dissolved in a 4 mM solution of PPTS in 200-proof EtOH (1.75 mL, 0.007 mmol). The resulting solution was stirred under ambient atmosphere in a Teflon-capped vial for 110 min and was then diluted with Et_2O (1.5 mL), petroleum ether (1.5 mL), and H_2O (3 mL). The phases were separated, and the aqueous phase was extracted with 1:1 petroleum ether:Et_2O (5 x 1.5 mL). The combined organic phase was dried over $MgSO_4$, filtered, and concentrated. Purification of the crude residue via silica gel chromatography (5 → 7% EtOAc in pentane) gave 37.2 mg of bryopyran **21** (90%) as a colorless residue. Compound purity was established by TLC (one spot) and ^{1}H-NMR analysis. Spectral data for **21** was identical to that observed previously.

Characterization data for **21**:

^{1}H-NMR (500 MHz, C_6D_6): δ 7.93 (2H, dd, *J* = 1.4, 8.1 Hz, -OTBDPS), 7.84-7.79 (2H, m, -OTBDPS), 7.37-7.21 (6H, m, -OTBDPS), 6.43 (1H, d, *J* = 1.9 Hz, C27), 6.30 (1H, d, *J* = 15.9 Hz, C17), 5.69 (1H, dd, *J* = 8.6, 15.9 Hz, C16), 5.68-5.62 (1H, m, C25), 5.61 (1H, s, C20), 4.86 (1H, d, *J* = 1.6 Hz, C38), 4.80 (1H, d, *J* = 1.4 Hz, C38), 4.84-4.79 (1H, m, C3), 4.43 (1H, tt, *J* = 2.2, 11.4 Hz, C23), 4.32 (1H, dd, *J* = 2.5, 13.9 Hz, C22), 4.20 (1H, ddd, *J* = 2.2, 8.6, 11.3 Hz, C15), 4.10 (1H, dd, *J* = 4.0, 9.6 Hz, C26), 3.57 (1H, dd, *J* = 8.6, 9.6, Hz, C26), 3.49-3.42 (1H, m, C11), 3.44 (1H, s, C19OH), 3.30 (3H, s, -OMe), 3.00 (1H, dd, *J* = 4.8, 11.2 Hz, C7), 2.82 (1H, dd, *J* = 2.1, 15.2 Hz, C2), 2.63-2.56 (2H, m, C2, C5), 2.47-2.39 (2H, m, C9, C12), 2.36-2.27 (2H, m, C12, C14), 2.16 (1H, dd, J = 13.0, 33.9 Hz, C14), 2.11-1.98 (3H, m, C31, C24), 1.94-1.71 (3H, m, C10, C22, C24), 1.64-1.43 (3H, m, C10, C32), 1.54 (3H, s, -C(CH_3)$_2$), 1.41-1.31 (2H, m, C4, C6), 1.23 (9H, s, -OTBDPS), 1.20-1.02 (8H, m, C4, C6, C33, C34, C35), 1.16 (3H, s, -C(CH_3)$_2$), 0.97 (9H, s, -OTBDMS), 0.95 (9H, s, -OTBDMS), 0.89 (3H, t, J = 7.1 Hz, C37), 0.92-0.84 (2H, m, C36), 0.82 (3H, s, -C(CH_3)$_2$), 0.74 (3H, s, -C(CH_3)$_2$), 0.11 (3H, s, -OTBDMS), 0.07 (3H, s, -OTBDMS), 0.05 (3H, s, -OTBDMS), -0.02 (3H, s, -OTBDMS) ppm.

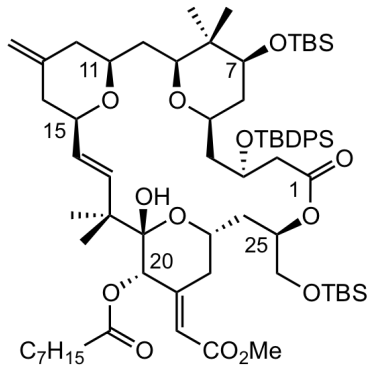
^{13}C-NMR (125 MHz, C_6D_6): δ 172.2, 171.7, 166.6, 152.0, 144.3, 138.9, 136.5, 136.3, 135.5 (2C), 134.4 (2C), 133.2, 130.07 (2C), 129.99 (2C), 120.4, 109.2, 98.5, 81.8, 80.8, 77.0, 76.4, 74.9, 74.3, 71.5, 70.1, 65.8, 65.0, 50.8, 45.55, 45.44, 44.3, 41.8, 41.1, 39.0, 38.5, 37.91, 37.90, 37.83, 34.7, 32.0, 29.30, 29.27, 27.3 (3C), 26.14 (3C), 26.08 (3C), 25.1, 24.6, 22.99, 22.91, 19.89, 19.71, 18.5, 18.3, 14.3, 12.9, -3.7, -4.77, -4.95, -5.11 (2 TBDPS carbons obstructed by C_6D_6 signal) ppm.

IR (**film**): 3510, 2955, 2929, 2856, 1734, 1723, 1472, 1463, 1428, 1388, 1361, 1251, 1228, 1157, 1102, 1059, 1004, 985, 836, 774, 702 cm^{-1}.

HRMS (TOF MS ES^+): calculated for $C_{70}H_{112}O_{13}Si_3Na$: 1267.7309; Found: 1267.7330.

$[\alpha]_D^{22.8}$ = -10.6 ^{o} (c = 0.6, CH_2Cl_2).

$\mathbf{R}_f$ = 0.43 (8% EtOAc: pentane) – one black spot, *p*-anisaldehyde stain.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7
O
O
15
OTBDPS
O
1
OH
O
O
25
20
OTBS
O
C_7H_{15}
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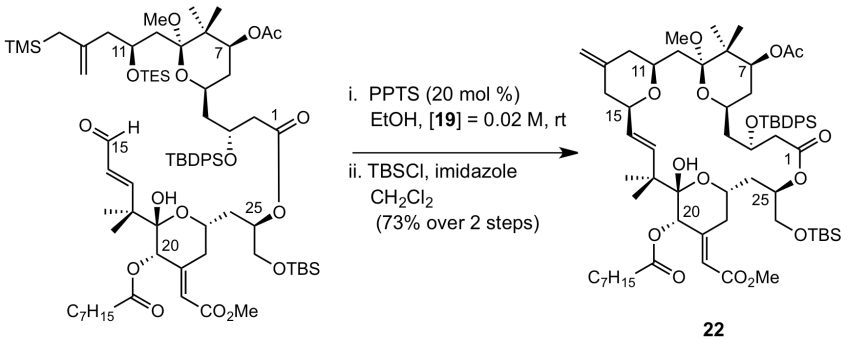
ppm 9
8
7
6
5
4
3
2
1
0



Procedure for bryopyran 22

Under ambient atmosphere, macrocyclization precursor **19** (12.5 mg, 0.00888 mmol) was dissolved in a solution of PPTS in anhydrous MeOH (4 mM, 443 μL, 0.0018 mmol). The vessel was sealed with a Teflon-lined cap and the reaction was stirred for 16 h. The reaction was then diluted with H_2O (1 mL), brine (1 mL), and Et_2O (1 mL). The phases were separated, and the aqueous phase was extracted with Et_2O (5 x 1 mL). The combined organic phase was dried over $MgSO_4$, filtered, and concentrated to give a crude residue that was used immediately in the next step.

The crude residue from above was dissolved in CH_2Cl_2 (900 μL). Imidazole (10.1 mg, 0.148 mmol) was added in one portion. Once all solids had dissolved, a solution of TBSCl in CH_2Cl_2 (0.97 M, 46 μL, 0.045 mmol) was added in one portion. After 2 h, the reaction was quenched with saturated aqueous NH_4Cl (2 mL). The biphasic mixture was diluted with Et_2O (2 mL), and the phases were separated. The aqueous phase was extracted with Et_2O (5 x 1.5 mL), and the combined organics were dried over $MgSO_4$, filtered, and concentrated to give a crude residue. Purification by silica gel chromatography (10% EtOAc in hexanes) gave 7.8 mg of bryopyran **22** (73%) as a colorless residue. Compound purity was established by TLC (one spot) and ^{1}H-NMR analysis.

Characterization data for **22**:

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.67 (m, 2H, -TBDPS), 7.61 (m, 2H, -TBDPS), 7.45-7.33 (m, 6H, -TBDPS), 6.00 (d, 1H, *J* = 1.8 Hz, C34), 5.92 (d, 1H, *J* = 16.0 Hz, C17), 5.53 (dd, 1H, *J* = 7.1, 16.0 Hz, C16), 5.23 (m, 1H, C25), 5.18 (dd, 1H, *J* = 5.0, 11.7 Hz, C7), 5.13 (s, 1H, C20), 4.79 (bs, 2H, C30), 4.56 (m, 1H, C3), 3.95 (ddd, 1H, *J* = 1.8, 7.1, 10.6 Hz, C15), 3.81 (m, 1H, C5), 3.73 (m, 1H, C23), 3.70 (s, 3H, -CO_2Me), 3.62 (dd, 1H, *J* = 2.3, 14.0 Hz, C22), 3.54-3.43 (m, 3H, C11, C26, C26), 2.69 (s, 3H, C9-OMe), 2.63 (dd, 1H, *J* = 4.1, 16.9 Hz, C2), 2.56 (s, 1H, C19-OH), 2.35-2.22 (m, 3H, C2, C40, C40), 2.13 (bd, 1H, *J* = 13.4 Hz, C14), 2.08 (bd, 1H, *J* = 13.1 Hz, C12), 2.04 (s, 3H, C7-OAc), 2.03-1.91 (m, 3H, C10, C14, C22), 1.88 (bt, 1H, *J* = 12.3 Hz, C12), 1.74-1.66 (m, 2H, C24, C24), 1.64-1.55 (m, 3H, C6, C41, C41), 1.54-1.40 (m, 3H, C4, C4, C10), 1.34-1.20 (m, 9H, C6, C42-C45), 1.17 (s, 3H, -CH_3), 1.01 (s, 9H, -TBDPS), 1.00 (s, 3H, -CH_3), 0.92 (s, 3H, -CH_3), 0.87 (t, 3H, *J* = 6.9 Hz, C46), 0.82 (s, 9H, -TBS), 0.81 (s, 3H, -CH_3), -0.08 (s, 3H, -TBS), -0.09 (s, 3H, -TBS) ppm.

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 172.2, 171.0, 170.2, 167.0, 151.5, 145.0, 135.9, 135.7, 135.1, 134.9, 134.2, 134.0, 129.8, 129.8, 127.8, 127.8, 120.0, 108.7, 103.0, 98.0, 78.4, 74.2, 74.1, 73.1, 69.8, 67.4, 65.4,

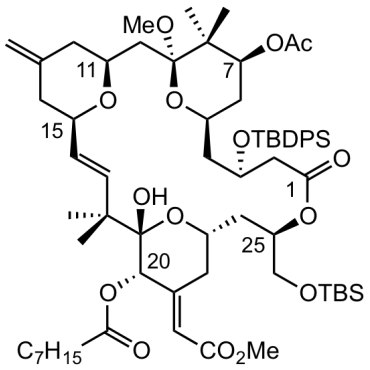
65.0, 64.6, 51.3, 48.0, 45.1, 44.9, 43.0, 41.7, 41.4, 40.7, 39.7, 37.2, 34.7, 33.6, 31.8, 31.2, 29.1, 29.0, 27.1, 25.9, 24.8, 24.1, 22.7, 21.5, 20.4, 20.2, 19.4, 18.4, 17.4, 14.2, -5.2, -5.3 ppm.

IR: 3513, 3071, 2932, 2858, 1738, 1666, 1471, 1428, 1386, 1365, 1247, 1161, 1104, 1025, 1004, 985, 886, 837, 777, 740, 704 cm^{-1}

HRMS (TOF MS ES+): Calculated for: $C_{67}H_{102}O_{15}Si_2Na^+$: 1225.6649; Found: 1225.6678.

$[\alpha]_D^{22.4}$ = + 10.8 ± 0.9 ° (*c* = 0.54, CH_2Cl_2)

$\mathbf{R}_f$ = 0.46 (15% EtOAc in petroleum ether), one purple spot, *p*-anisaldehdye stain.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OAc
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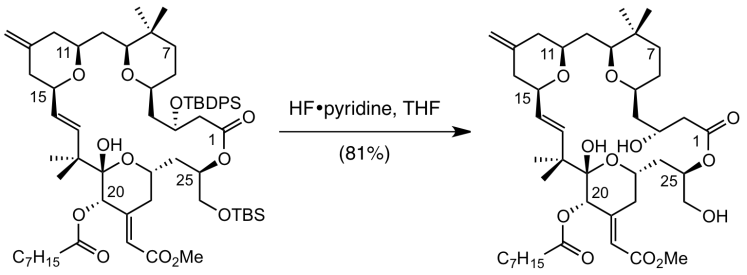
7
O
O
15
OTBDPS
O
1
OH
O
O
25
20
OTBS
O
C_7H_{15}
O
CO_2Me

22

8
7
6
5
4
3
2
1
0
ppm



20 **1**

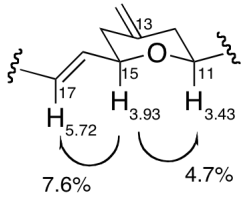
Procedure for analog 1

In a polypropylene vial, bryopyran **20** was dissolved in THF (1.4 mL) under N_2. The resulting solution was cooled in an ice water bath. HF•pyridine (70% HF, 250 μL) was added dropwise over 1 min. After stirring 10 minutes, the cold bath was removed, and the solution was stirred an additional 96 hours at ambeint temperature. The reaction mixture was then quenched into saturated aqueous $NaHCO_3$ (15 mL) and was diluted with EtOAc (25 mL). The layers were partitioned, and the aqueous layer was extracted with EtOAc (3 x 25 ml). The organic phase was dried over Na_2SO_4, filtered and concentrated. Purification by silica gel chromatography (35→40% EtOAc:Pentane) followed by C18 reverse-phase high pressure liquid chromatography (65% MeCN:H_2O → 100% MeCN) afforded pure analog **1** (2.0 mg, 81%) as an amorphous white solid.

Characterization data for ester **1**:

^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.99 (d, *J* = 0.1 Hz, 1H, C27), 5.72 (d, *J* = 15.8 Hz, 1H, C17), 5.37 (m, 1H, C25), 5.33 (dd, *J* = 8.4, 15.9 Hz, 1H, C16), 5.20 (s, 1H, C19-OH), 5.14 (s, 1H, C20), 4.73 (m, 2H, C30), 4.49 (d, *J* = 12.1 Hz, 1H, C3-OH), 4.22 (m, 1H, C3), 4.08 (tt, *J* = 2.1, 11.4 Hz, 1H, C23), 3.93 (ddd, *J* = 2.3, 8.4, 11.0 Hz, 1H, C15), 3.84 (ddd, *J* = 3.2, 4.7, 12.1 Hz, 1H, C26), 3.71 (dd, *J* = 2.2, 13.8 Hz, 1H, C22), 3.68 (s, 3H, -CO_2Me), 3.65 (m, 1H, C26), 3.48 (m, 1H, C5), 3.43 (ddd, *J* = 2.1, 6.0, 11.0 Hz, 1H, C11), 3.13 (dd, *J* = 1.9, 11.6 Hz, 1H, C9), 2.47 (m, 2H, C2), 2.30 (m, 2H, C31), 1.89-2.17 (m, 8H, C4 & C12 & C12 & C14 & C14 & C22 & C24 & C26-OH), 1.79 (ddd, *J* = 2.6, 11.7, 14.0 Hz, 1H, C24), 1.47-1.71 (m, 3H, C6 & C6 & C10), 1.39-1.57 (m, 4H, C4, C10, C32, C32), 1.19-1.39 (m, 10H, C7 & C7 & C33-36), 1.14 (s, 3H, C28), 1.01 (s, 3H, C29), 0.87 (t, *J* = 6.9 Hz, 3H, C37), 0.86 (s, 3H, -C(8)$(CH_3)_2$), 0.80 (s, 3H, -C(8)$(CH_3)_2$) ppm.

1D nOe ($CDCl_3$, 400 MHz)*: Assignment of relative C11-C15 stereochemistry:



*nOe enhancements reported relative to the irradiated signal strength set at -100%

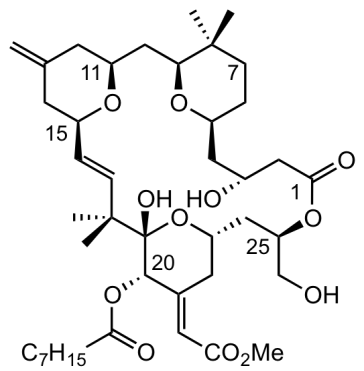
^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.7, 172.4, 167.3, 152.1, 144.3, 138.2, 130.5, 120.0, 108.7, 99.1, 86.5, 80.1, 78.2, 76.3, 74.6, 71.7, 68.9, 66.0, 64.7, 51.3, 45.0, 43.1, 42.7, 41.5, 40.5, 39.1, 37.1, 36.1, 34.9, 32.5, 31.9, 31.3, 29.3, 29.2, 29.2, 27.9, 25.0, 24.9, 22.8, 20.0, 19.5, 14.3 ppm.

IR (film): 3449, 3333, 2929, 2852, 1736, 1720, 1664, 1432, 1406, 1364, 1323, 1281, 1254, 1229, 1157, 1099, 1085, 1048, 1024, 1006, 884, 732 cm^{-1}.

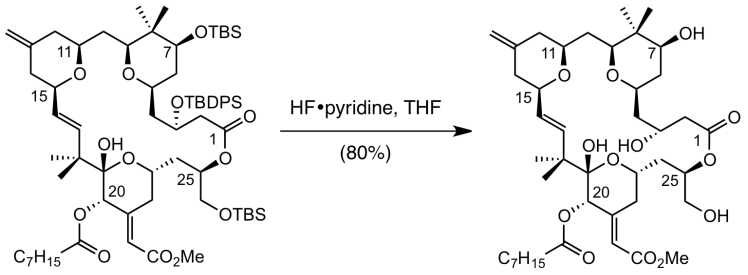
HRMS (TOF MS ES+): Calculated for $C_{42}H_{66}O_{12}Na^{+}$: 785.4452; Found: 785.4447.

$[\alpha]_D^{25}$ = -3.30° (*c* = 0.20, $CHCl_3$).

$\mathbf{R}_f$ = 0.38 (50% EtOAc , 50% Pentane) – one black spot (*p*-anisaldehyde stain).



1



21 **2**

Procedure for analog 2

Bryopyran **21** (3.5 mg, 0.003 mmol) was dissolved in THF (2.8 mL) in a polypropylene vial charged with a Teflon-coated magnetic stir bar under positive pressure of N_2 gas. The solution was cooled to –78 ^oC in a CO_2/acetone bath, and HF•pyridine (711 mL, 70% HF, 30% pyridine, Aldrich) was added dropwise via syringe over 30 s. After 20 min, the reaction was allowed to warm to room temperature. After 21 h, the reaction was added portionwise to saturated $NaHCO_3$ (17.5 mL) and diluted with H_2O (3.5 mL) and EtOAc (14 mL). The organic layer was separated, and the aqueous layer was washed with EtOAc (3 x 18 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated *in vacuo*. Flash chromatography on silica (80% EtOAc: Pentane) provided 1.8 mg (80%) of **2** as a white solid.

Characterization data for ester **2**:

^{1}H-NMR (600 MHz, $CDCl_3$): δ 5.99 (1H, d, *J* = 1.8 Hz, C27), 5.72 (1H, d, *J* = 15.8 Hz, C17), 5.40-5.35 (1H, m, C25), 5.32 (1H, dd, *J* = 8.4, 15.9 Hz, C16), 5.16 (1H, s, C20), 5.14 (1H, s, C19OH), 4.74 (1H, d, *J* = 1.5 Hz, C38), 4.73 (1H, d, *J* = 1.3 Hz, C38), 4.42 (1H, d, *J* = 11.9 Hz, C3OH), 4.24 (1H, m, C3), 4.07 (1H, tt, *J* = 2.6, 13.9 Hz, C23), 3.93 (1H, ddd, *J* = 2.5, 8.3, 11.3 Hz, C15), 3.84 (1H, dd, *J* = 3.1, 12.0 Hz, C26), 3.72 (1H, dd, *J* = 2.2, 13.8 Hz, C22), 3.68 (3H, s, -OMe), 3.64 (1H, dd, *J* = 5.8, 11.9 Hz, C26), 3.63-3.58 (1H, m, C11), 3.45-3.41 (1H, m, C5), 3.37 (1H, dd, *J* = 4.6, 11.6 Hz, C9), 3.07 (1H, dd, *J* = 2.1, 11.7 Hz, C7), 2.51-2.42 (2H, m, C2), 2.35-2.25 (2H, m, C31), 2.16-1.92 (8H, C12, C14, C22, C24, C7OH, C26OH), 1.82-1.72 (2H, m, C6, C24), 1.68-1.47 (6H, m, C4, C6, C10, C32), 1.43 (1H, q, *J* = 11.8 Hz, C10), 1.32-1.20 (8H, m, C33, C34, C35, C36), 1.14 (3H, s, -$C(CH_3)_2$), 1.01 (3H, s, -$C(CH_3)_2$), 0.92 (3H, s, -$C(CH_3)_2$), 0.87 (3H, t, *J* = 6.9 Hz, C37), 0.81 (3H, s, -$C(CH_3)_2$) ppm.

^{13}C-NMR (125 MHz, C_6D_6): δ 172.1, 171.7, 166.8, 152.6, 144.5, 138.7, 130.8, 120.5, 108.6, 99.7, 84.8, 80.3, 77.8, 75.0, 74.6, 73.8, 71.7, 68.9, 65.72, 65.16, 50.6, 45.4, 42.91, 42.80, 41.8, 39.8, 38.8, 37.4, 36.28, 36.21, 34.8, 32.0, 31.9, 29.34, 29.28, 25.35, 25.14, 22.97, 22.39, 20.0, 14.3, 12.6 ppm.

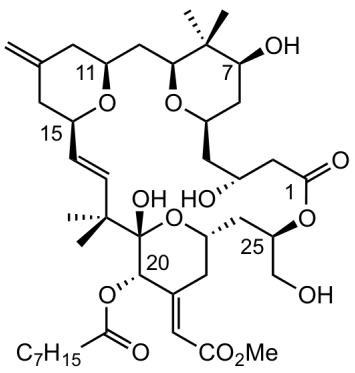
IR (**film**): 3445, 3355, 2927, 2855, 1727, 1665, 1407, 1362, 1260, 1230, 1153, 1085, 1048, 1025, 1003, 984, 877, 803 cm^{-1}.

HRMS (TOF MS ES^+): calculated for $C_{42}H_{66}O_{13}Na$: 801.4401; Found: 801.4413.

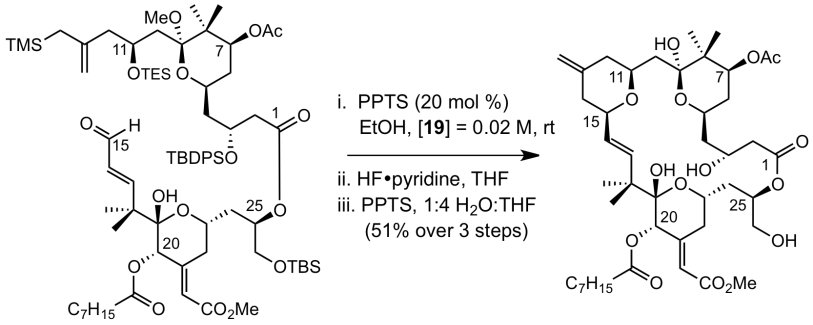
$[\alpha]_D^{22.8}$ = -11.6 ^{o} (c = 0.4, CH_2Cl_2).

R_f = 0.34 (80% EtOAc: pentane) – one black spot, *p*-anisaldehyde stain.

HPLC: Retention Time = 18.52 min. Method: Semi-prep column; 65% - 95% MeCN in H_2O at 6 mL/min over 30 min.



2



Procedure for analog 4

To neat ester **19** (7.2 mg, 0.0051 mmol) was added a solution of PPTS in anhydrous MeOH (4 mM, 260 µL, 0.0010 mmol). The resulting mixture was stirred in a Teflon-capped vial for 19 h and was diluted with H_2O (250 µL), Et_2O (250 µL), and brine (250 µL). The phases were separated, and the aqueous layer was extracted with Et_2O (4 x 500 µL). The combined organic phase was dried over $MgSO_4$, filtered, and concentrated to afford a crude residue that was used directly in the subsequent step.

In a polypropylene vial, the crude residue (max. 0.0051 mmol) from the previous step was dissolved in THF (2.0 mL) and was cooled to 0 ºC in an ice water bath. HF·pyridine (70% HF, 500 µL) was added dropwise via syringe over 30 s, and the reaction vessel was sealed with a screw-cap. The reaction was warmed to ambient temperature and was stirred for 98 h, at which time it was quenched by its dropwise addition to stirred, saturated aqueous $NaHCO_3$ (25 mL). The resulting pH ~7 mixture was diluted with EtOAc (25 mL), and the phases were separated. The aqueous phase was extracted with EtOAc (2 x 20 mL), and the combined organic phase was washed with 0.2 N HCl (30 mL). The acidic aqueous phase was extracted with EtOAc (2 x 20 mL), and the combined organic phase was dried over $MgSO_4$, filtered, and concentrated to afford a crude residue that was used without further purification.

The residue from the previous step (max. 0.0051 mmol) was dissolved in a 0.05 M PPTS solution in 1:4 H_2O:THF (600 µL, 5.9 mmol). The resulting solution was stirred for 44 h at ambient temperature and was diluted with Et_2O (1 mL) and H_2O (2 mL). The phases were separated, and the aqueous phase was extracted with Et_2O (5 x 1 mL). The combined organic phase was dried over $MgSO_4$, filtered, and concentrated to a crude residue that was purified by silica gel chromatography (55% EtOAc in hexanes) and reverse-phase chromatography (C18, 10 micron, 10 x 250 mm, 65 → 100 % MeCN in H_2O) to afford 2.2 mg of analog **4** (51% over three steps) as an amorphous white solid following lyophilization. Compound purity was established by TLC (one spot) and ^{1}H-NMR analysis.

Characterization data for ester **4**:

^{1}H NMR ($CDCl_3$, 600 MHz): δ = 5.98 (d, 1H, *J* = 1.7 Hz, C34), 5.73 (d, 1H, *J* = 15.8 Hz, C17), 5.34 (m, 1H, C25), 5.31 (dd, 1H, *J* = 8.3, 15.8 Hz, C16), 5.18 (s, 1H, C19-OH), 5.16 (dd, 1H, *J* = 4.8, 11.8 Hz), 5.13 (s, 1H, C20), 4.76 (bs, 1H, C30), 4.74 (bs, 1H, C30), 4.26 (d, 1H, *J* = 12.0 Hz, C3-OH), 4.26-4.15 (m, 2H, C3, C5), 4.05 (tt, 1H, *J* = 2.1, 11.3 Hz, C23), 3.94 (ddd, 1H, 2.3, 8.5, 11.0 Hz, C15), 3.84 (bd, 1H, *J* = 12.0, C26), 3.70 (dd, 1H, *J* = 2.0, 13.6, C22), 3.68 (s, 3H, CO_2Me), 3.67-3.60 (m, 2H, C11, C26), 2.49 (dd, 1H, *J*

= 3.1, 12.9 Hz, C2), 2.46 (dd, 1H, *J* = 10.9, 12.9 Hz, C2), 2.36 (s, 1H, C9-OH), 2.30 (m, 2H, C40, C40), 2.12-2.06 (m, 4H, C10, C12, C12, C14), 2.05 (s, 3H, C7-OAc), 2.05-1.95 (m, 4H, C4, C14, C22, C24), 1.79 (ddd, 1H, *J* = 2.5, 11.6, 14.1 Hz, C24), 1.77 (ddd, 1H, 2.8, 4.8, 12.4 Hz, C6), 1.67 (d, 1H, *J* = 15.1 Hz, C10), 1.63-1.57 (m, 3H, *J* = C4, C41, C41), 1.47 (q, 1H, *J* = 12.0 Hz, C6), 1.31-1.22 (m, 8H, C42-C45), 1.15 (s, 3H, C32), 1.00 (bs, 6H, C29, C33), 0.95 (s, 3H, C28), 0.87 (bt, 3H, *J* = 7.0 Hz, C46) ppm.

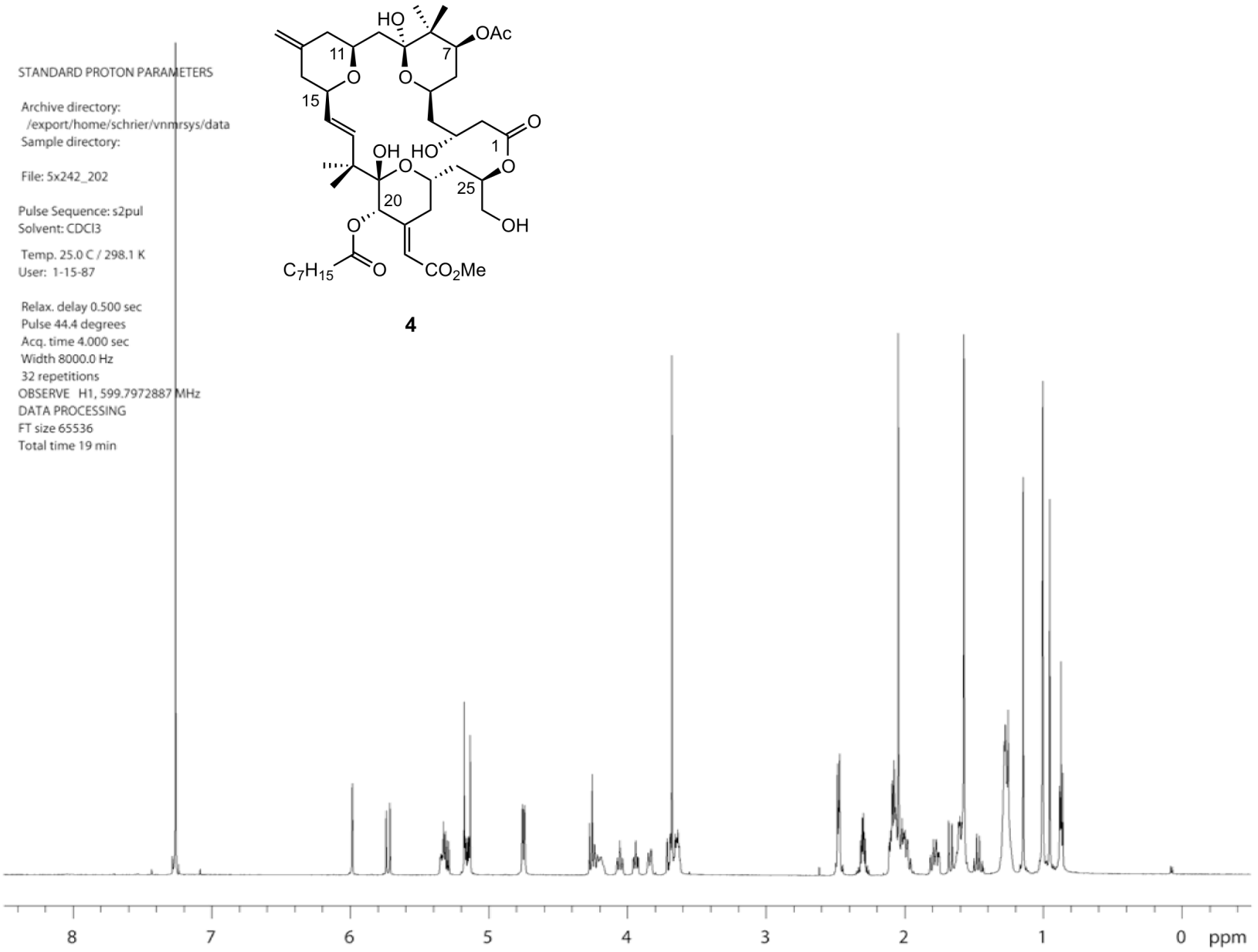
^{13}C NMR ($CDCl_3$, 125 MHz): δ = 172.5, 172.3, 171.0, 167.1, 151.8, 143.5, 138.6, 130.0, 120.0, 109.2, 102.0, 99.0, 80.3, 74.4, 73.0, 72.1, 71.8, 68.5, 65.9, 65.7, 64.7, 51.3, 45.0, 42.7, 42.5, 42.1, 41.3, 41.0, 39.9, 36.0, 34.8, 33.5, 31.8, 31.2, 29.2, 29.0, 24.8, 24.8, 22.7, 21.3, 21.2, 19.9, 17.0, 14.2 ppm.

IR: 3459, 3338, 2931, 1738, 1722, 1715, 1667, 1434, 1408, 1366, 1246, 1156, 1079, 1028, 1003, 984, 890, 735 cm^{-1}

HRMS: (TOF MS ES^+): Calculated for $C_{44}H_{68}O_{15}Na^+$: 859.4450; Found: 859.4456.

$[\alpha]_D^{23.5}$ = + 17.6 ± 3.2 º (*c* = 0.15, CH_2Cl_2)

R_f = 0.26 (50% EtOAc in petroleum ether), one purple spot, *p*-anisaldehyde stain.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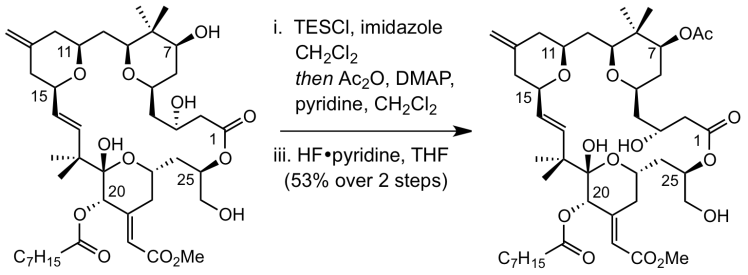
4



2 **3**

Procedure for analog 3

Analog **2** (1.1 mg, 0.001 mmol) was dissolved in CH_2Cl_2 (0.1 mL) in an oven-dried, Teflon-capped vial charged with a Teflon-coated magnetic stir bar and purged with N_2. DMAP (0.2 mg, 0.002 mmol, Aldrich) was added in one portion, and the solution was cooled to –10 ^oC in an ice/acetone bath (temperature monitored externally). TESCl (0.26 μL, 0.002 mmol, Aldrich) was then added in one portion via microsyringe. After 15 min, appreciable starting material remained by TLC, and the vial was allowed to warm to room temperature. DMAP and TESCl were subsequently added in 0.2 mg and 0.26 μL portions, respectively, until all of the starting material was consumed (monitored by TLC). In total, 7.2 equivalents of DMAP and 6.6 equivalents of TESCl were required for complete conversion of starting material. The reaction was quenched with saturated NH_4Cl (0.3 mL) and diluted with H_2O (0.5 mL) and Et_2O (1 mL). The organic layer was separated, and the aqueous layer was washed with Et_2O (4 x 1 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated. Flash chromatography on silica (43% EtOAC: Pentane) provided 0.9 mg (71%) of product as a colorless oil.

The C26 TES protected product (0.9 mg, 0.001 mmol) from above was dissolved in CH_2Cl_2 (0.1 mL) in a Teflon-capped, oven-dried vial purged with N_2 and charged with a Teflon-coated magnetic stir bar. Pyridine (0.15 μL, 0.002 mmol, Aldrich) was added in one portion via microsyringe, followed by the addition of DMAP (0.2 mg, 0.002 mmol, Aldrich) in one portion. Next, Ac_2O (0.15 μL, 0.002 mmol, Aldrich) was added in one portion via microsyringe. The reaction was stirred at room temperature for 2 h, at which point it was quenched with saturated NH_4Cl (0.3 mL) and diluted with H_2O (0.2 mL) and Et_2O (1 mL). The organic layer was separated, and the aqueous layer was washed with Et_2O (4 x 1 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated. Flash chromatography on silica (15% EtOAc: Pentane) provided 0.7 mg (74%) of product as a colorless oil.

The acylated product from above (0.8 mg, 0.0009 mmol) was dissolved in THF (0.86 mL) in a polypropylene vial equipped with a Teflon-coated magnetic spin vane and under positive pressure of N_2 gas. The solution was cooled to –78 ^oC in a CO_2/acetone bath, and HF•pyridine (108 μL, 70% HF, 30% pyridine, Aldrich) was added dropwise via syringe over 15 s. After 20 min, the reaction was allowed to warm to room temperature. After 12 h, the reaction was transferred to saturated $NaHCO_3$ (5 mL) and diluted with H_2O (1 mL) and EtOAc (4 mL). The organic layer was separated, and the aqueous layer was washed with EtOAc (3 x 5 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated *in vacuo*. Flash chromatography on silica (60% EtOAc: Pentane) provided 0.7 mg (100%) of **3** as a white solid.

Characterization data for ester **3**:

^{1}H-NMR (600 MHz, $CDCl_3$): δ 5.98 (1H, d, *J* = 1.8 Hz, C27), 5.72 (1H, d, *J* = 15.8 Hz, C17), 5.40-5.35 (1H, m, C25), 5.32 (1H, dd, *J* = 8.3, 15.8 Hz, C16), 5.16 (1H, s, C20), 5.14 (1H, s, C19OH), 4.75 (1H, d, *J* = 1.5 Hz, C38), 4.73 (1H, d, *J* = 1.3 Hz, C38), 4.57 (1H, dd, *J* = 4.7, 11.6 Hz, C7), 4.39 (1H, d, J = 11.8 Hz, C3OH), 4.24 (1H, m, C3), 4.06 (1H, tt, *J* = 2.2, 11.3 Hz, C23), 3.94 (1H, ddd, J = 2.6, 8.4, 11.3 Hz, C15), 3.84 (1H, dd, *J* = 3.1, 12.0 Hz, C26), 3.71 (1H, dd, J = 2.3, 13.9 Hz, C22), 3.71-3.62 (1H, m, C11), 3.68 (3H, s, -OMe), 3.64 (1H, dd, J = 5.7, 12.0 Hz, C26), 3.47-3.42 (1H, m, C5), 3.17 (1H, dd, *J* = 2.1, 11.6 Hz, C9), 2.50-2.41 (2H, m, C2), 2.35-2.25 (2H, m, C31), 2.16-1.93 (7H, C12, C14, C22, C24, C26OH), 2.05 (3H, s, $-C(O)CH_3$), 1.82-1.70 (3H, m, C6, C24), 1.67-1.44 (6H, m, C4, C10, C32), 1.32-1.21 (8H, m, C33, C34, C35, C36), 1.14 (3H, s, $-C(CH_3)_2$), 1.01 (3H, s, $-C(CH_3)_2$), 0.91 (3H, s, $-C(CH_3)_2$), 0.87 (3H, t, *J* = 6.8 Hz, C37), 0.82 (3H, s, $-C(CH_3)_2$) ppm.

^{13}C-NMR (125 MHz, C_6D_6): δ 172.0, 171.7, 169.8, 166.7, 152.7, 144.3, 138.9, 130.7, 120.5, 108.7, 99.7, 84.5, 80.3, 77.6, 76.3, 75.0, 73.5, 71.8, 68.8, 65.7, 65.2, 50.6, 45.4, 42.8, 41.8, 39.7, 37.5, 36.21, 36.13, 34.8, 34.0, 32.03, 31.88, 30.2, 29.35, 29.29, 25.3, 25.1, 23.0, 22.3, 20.6, 20.0, 14.3, 13.8 ppm.

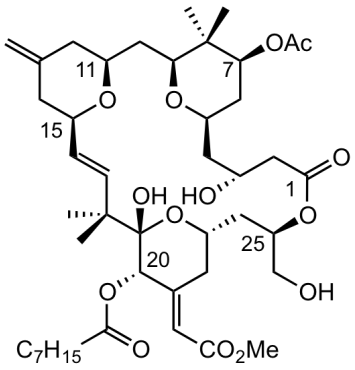
IR (**film**): 3459, 3350, 2928, 2854, 1735, 1730, 1664, 1433, 1363, 1278, 1244, 1153, 1097, 1083, 1047, 1026, 1004, 884 cm^{-1}.

HRMS (TOF MS ES^+): calculated for $C_{44}H_{68}O_{14}Na$: 843.4507; Found: 843.4507.

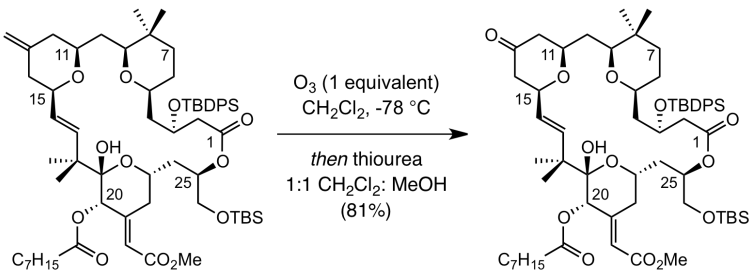
$[\alpha]_D^{22.8}$ = 4.7 ° (c = 0.4, CH_2Cl_2).

R_f = 0.28 (60% EtOAc: pentane) – one black spot, *p*-anisaldehyde stain.

HPLC: Retention Time = 23.24 min. Method: Semi-prep column; 65% - 95% MeCN in H_2O at 6 mL/min over 30 min.



3



20 **23**

Procedure for ketone 23

A 25 mL flask was charged with a magnetic stirbar and 12 mL CH_2Cl_2. Under ambient atmosphere, the system was cooled in a CO_2/acetone bath. Once cold (~5 min), ozone gas was bubbled through the CH_2Cl_2 for 5 min, resulting in a deep blue ozone solution (ca. 0.025 M). The reaction vessel was capped, flushed with N_2, and stored at -78 °C for immediate use.

A solution of pyran **20** (5.2 mg, 0.0047 mmol) in CH_2Cl_2 (160 μL) under ambient atmosphere was cooled in a CO_2/acetone bath. To this solution was added an aliquot of the above described ozone solution (ca. 0.025 M, 385 μL, 0.0096 mmol) via disposable polypropylene syringe. The resulting solution was stirred 5 minutes, after which, TLC analysis demonstrated consumption of starting material. Thiourea (7.1 mg, 0.098 mmol) was then added, followed by MeOH (0.65 mL). The resulting mixture was allowed to slowly warm to ambient temperature, by which time all of the thiourea had dissolved. The resulting solution was stirred 36 h at ambient temperature, at which time TLC analysis demonstrated convergence to one major product. The reaction solution was concentrated under a steady stream of N_2 to afford a crude material that was loaded directly onto a silica gel column and purified via silica gel chromatography (10→12→14→16→18% EtOAc:Pentane) to provide desired ketone **23** (4.2 mg, 81%) as a clear oil.

Characterization data for ketone **23**:

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.68-7.76 (m, 4H, -SiPh), 7.34-7.40 (m, 6H, -SiPh), 6.00 (d, *J* = 1.6 Hz, 1H, C27), 5.98 (d, *J* = 15.9 Hz, 1H, C17), 5.55, (dd, *J* = 8.4, 15.9 Hz, 1H, C16), 5.19-5.31 (m, 1H, C25), 5.14 (s, 1H, C20), 4.37-4.46 (m, 1H, C3), 4.17 (ddd, *J* = 3.1, 8.9, 11.35, 1H, C15), 3.95 (bt, *J* = 11.1 Hz, 1H, C23), 3.80 (dd, *J* = 3.9, 10.1 H, 1H, C26), 3.69 (s, 3H, -CO_2Me), 3.68-3.72 (m, 1H, C22), 3.47 (dd, *J* = 7.7, 10.0 Hz, 1H, C26), 3.37 (bt, *J* = 10.1 Hz, 1H, C11), 2.73 (s, 1H, C19-OH), 2.49-2.62 (m, 2H, C2 & C2), 2.22-2.43 (m, 7H, C5 & C9 & C12 & C14 & C14 & C30 & C30), 1.96-2.14 (3H, C12 & C22 & C24), 1.84 (ddd, *J* = 1.7, 11.6, 13.8 Hz, 1H, C24), 1.62 (m, 2H, C31), 1.24-1.51 (m, 16H, C4 & C6 & C7 & C10 & C32-35), 1.23 (s, 3H, C28), 1.05 (s, 3H, C29), 0.97 (s, 9H, , -Si^{t}Bu), 0.91 (s, 9H, , -Si^{t}Bu), 0.88 (t, *J* = 7.1 Hz, 3H, C36), 0.67 (s, 3H, C(8)(CH_3)$_2$), 0.62 (s, 3H, C(8)(CH_3)$_2$), 0.09 (s, 3H, -SiMe), 0.06 (s, 3H, -SiMe) ppm.

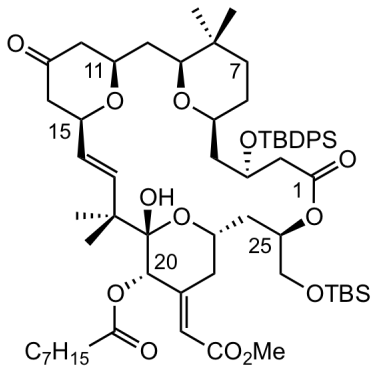
^{13}C NMR ($CDCl_3$, 125 MHz): δ 206.5, 172.3, 172.1, 166.7, 151.4, 139.3, 136.2, 136.0, 134.8, 133.5, 131.4, 130.1, 129.6, 127.9, 127.6, 119.7, 97.9, 81.9, 78.4, 76.1, 74.9, 74.2, 70.6, 69.6, 65.5, 64.5, 51.4, 58.4, 47.7, 45.4, 45.2, 44.4, 38.7, 38.6, 37.2, 34.8, 31.8, 31.4, 29.9, 29.2, 29.0, 28.4, 27.1, 27.0, 26.0, 24.8, 24.0, 22.7, 19.7, 19.4, 18.7, 18.4, 14.2, -5.0, -5.1 ppm.

IR (film) 3518, 2928, 2855, 1723, 1667, 1471, 1429, 1388, 1362, 1257, 1228, 1158, 1105, 1004, 837, 778, 704, 665 cm^{-1}.

HRMS (TOF MS ES+): Calculated for $C_{63}H_{96}O_{13}Si_2Na^+$: 1139.6287; Found: 1139.6280.

$[\alpha]_D^{25}$ = -10.79° (*c* = 0.42, $CHCl_3$).

$\mathbf{R}_f$ = 0.42 (20% EtOAc , 80% Pentane) – one black spot (*p*-anisaldehyde stain).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0
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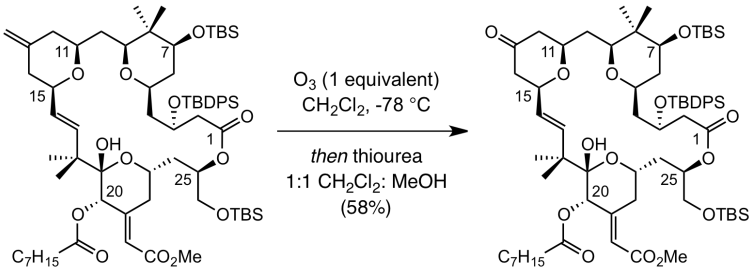


21 **24**

Procedure for ketone 24

Bryopyran **21** (20.3 mg, 0.016 mmol) was dissolved in CH_2Cl_2 (1.25 mL) in a glass vial under ambient atmosphere charged with a Teflon-coated magnetic stir bar. The solution was cooled to –78 °C in a CO_2/acetone bath and an aliquot of ozone solution (ca 0.025 M in CH_2Cl_2) was added dropwise via syringe over 5 s. After 15 min, TLC analysis showed complete consumption of starting material, and thiourea (13.9 mg, 0.182 mmol, Aldrich) was added in one portion. Following thiourea addition, MeOH (1.25 mL) was added in one portion via syringe. After 15 min, the reaction was allowed to warm to room temperature and proceed for 19 h, at which point it was concentrated under a stream of N_2 gas. Flash chromatography on silica (10% EtOAc: Pentane) provided 11.8 mg (58%) of **24** as a colorless oil.

Characterization data for ketone **24**:

^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.75-7.70 (4H, m, -OTBDPS), 7.47-7.35 (6H, m, -OTBDPS), 5.99 (1H, d, *J* = 1.8 Hz, C27), 5.96 (1H, d, *J* = 15.9 Hz, C17), 5.54 (1H, dd, *J* = 8.5, 15.9 Hz, C16), 5.29-5.22 (1H, m, C25), 5.13 (1H, s, C20), 4.44-4.39 (1H, m, C3), 4.15 (1H, ddd, *J* = 2.6, 8.6, 11.4 Hz, C15), 3.95 (1H, tt, *J* = 2.2, 11.3 Hz, C23), 3.80 (1H, dd, *J* = 3.9, 10.2 Hz, C26), 3.71-3.67 (1H, m, C22), 3.69 (3H, s, -OMe), 3.49 (1H, dd, *J* = 7.6, 10.2 Hz, C26), 3.30-3.24 (1H, m, C11), 2.84 (1H, dd, *J* = 4.8, 11.2 Hz, C7), 2.73 (1H, s, C19OH), 2.60-2.50 (2H, m, C2), 2.46-2.39 (1H, m, C5), 2.39-2.25 (5H, C12, C14, C31), 2.18 (1H, dd, J = 0.7, 10.8 Hz, C9), 2.12-1.96 (3H, m, C12, C22, C24), 1.86 (1H, ddd, J = 2.0, 11.9, 14.6 Hz, C24), 1.68-1.51 (5H, C10, C32, C33), 1.42-1.21 (10H, C4, C6, C10, C34, C35, C36), 1.22 (3H, s, -$C(CH_3)_2$), 1.04 (3H, s, -$C(CH_3)_2$), 0.97 (9H, s, -OTBDPS), 0.91 (9H, s, -OTBDMS), 0.88 (3H, t, *J* = 6.9 Hz, C37), 0.85-0.71 (1H, m, C6), 0.82 (9H, s, -OTBDMS), 0.63 (3H, s, -$C(CH_3)_2$), 0.60 (3H, s, -$C(CH_3)_2$), 0.09 (3H, s, -OTBDMS), 0.06 (3H, s, -OTBDMS), -0.06 (3H, s, -OTBDMS), -0.10 (3H, s, -OTBDMS) ppm.

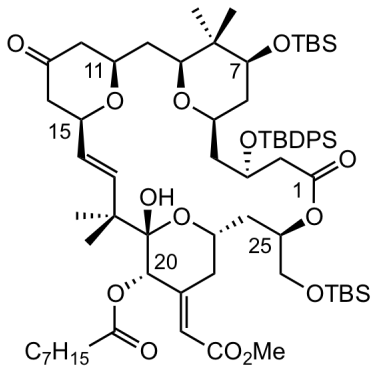
^{13}C-NMR (125 MHz, C_6D_6): δ 206.4, 172.3, 172.0, 166.7, 151.3, 139.0, 136.3 (2C), 135.9 (2C), 134.8, 133.5, 131.5, 130.2, 129.7, 127.9 (2C), 127.6 (2C), 119.7, 97.9, 80.7, 78.3, 75.8, 75.0, 74.3, 73.7, 70.4, 69.7, 65.6, 64.5, 51.3, 48.4, 47.7, 45.5, 45.05, 44.2, 38.6, 38.1, 37.4, 37.1, 34.8, 31.8, 31.3, 29.8, 29.17, 29.02, 27.0 (3C), 26.02 (3C), 25.89 (3C), 24.8, 24.1, 22.7, 19.7, 19.5, 18.4, 18.1, 14.2, 12.6, -3.8, -4.93, -5.05, -5.09 ppm.

IR (**film**): 2955, 2929, 2857, 1723, 1471, 1428, 1388, 1362, 1251, 1228, 1156, 1102, 1059, 1004, 986, 836, 775, 704 cm^{-1}.

HRMS (TOF MS ES^+): calculated for $C_{69}H_{110}O_{14}Si_3Na$: 1269.7101; Found: 1269.7109.

$[\alpha]_D^{22.8}$ = -14.8 ° (c = 1.1, CH_2Cl_2).

$\mathbf{R}_f$ = 0.29 (10% EtOAc: pentane) – one black spot, *p*-anisaldehyde stain.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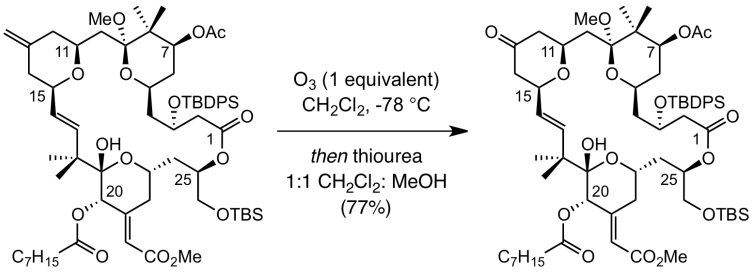


22 **25**

Procedure for ketone 25

A solution of O_3 in CH_2Cl_2 (ca. 25 mM) was prepared by bubbling O_3 (2 LPM) for ~5 min through CH_2Cl_2 (50 mL) in a CO_2:acetone bath at −78 ºC. The resulting bright blue solution was used immediately.

Olefin **22** (7.8 mg, 0.0065 mmol) was dissolved in CH_2Cl_2 (300 µL) under ambient atmosphere and was cooled to –78 °C in a CO_2:acetone bath. An aliquot of O_3 solution (~25 mM, 260 µL, ~0.0065 mmol) was added via plastic syringe in one portion. After 5 min, thiourea (10 mg, 0.13 mmol) was added in one portion followed by MeOH (560 µL). The resulting mixture was removed from the cold bath. On warming to ambient temperature, all solids dissolved and the solution was stirred for an additional 17 h. The reaction was concentrated under a stream of N_2 and was directly loaded onto a slurry-packed (17% EtOAc in pentane) silica gel column. Elution with 17% EtOAc in pentane provided 6.0 mg of ketone **25** (77%) as a colorless residue. Compound purity was established by TLC (single spot) and ^{1}H-NMR analysis.

Characterization data for ketone **25**:

^{1}H NMR ($CDCl_3$, 600 MHz): δ = 7.65 (m, 2H, -TBDPS), 7.59 (m, 2H, -TBDPS), 7.47-7.32 (m, 6H, -TBDPS), 6.00 (d, 1H, *J* = 2.0 Hz, C34), 5.99 (d, 1H, *J* = 16.1 Hz, C17), 5.55 (dd, 1H, *J* = 6.8, 16.1 Hz, C16), 5.22 (m, 1H, C25), 5.21 (dd, 1H, *J* = 4.8, 11.7 Hz, C7), 5.14 (s, 1H, C20), 4.48 (tt, 1H, *J* = 2.9, 9.8 Hz, C3), 4.28 (ddd, 1H, *J* = 2.2, 6.8, 11.2 Hz, C15), 3.91 (dddd, 1H, *J* = 2.7, 2.7, 8.9, 11.6 Hz, C5), 3.75 (ddd, 1H, *J* = 2.0, 9.0, 11.5 Hz, C11), 3.70 (s, 3H, -CO_2Me), 3.69 (m, 1H, C23), 3.62 (dd, 1H, *J* = 2.2, 13.9 Hz, C22), 3.48-3.43 (m, 2H, C26, C26), 2.68 (s, 3H, C9-OMe), 2.67 (dd, 1H, *J* = 3.5, 17.1 Hz, C2), 2.36 (s, 1H, C19-OH), 2.34-2.19 (m, 6H, C2, C12, C14, C14, C40, C40), 2.15 (dd, 1H, *J* = 12.0, 14.0 Hz, C12), 2.12 (dd, 1H, *J* = 8.9, 16.3 Hz, C10), 2.05 (s, 3H, C7-OAc), 2.00 (ddd, 1H, *J* = 2.0, 11.4, 13.7 Hz, C22), 1.73-1.65 (m, 3H, C6, C24, C24), 1.64-1.57 (m, 2H, C41, C41), 1.51 (d, 1H, *J* = 16.3 Hz, C10), 1.51-1.42 (m, 2H, C4, C4), 1.35 (*app*-q, 1H, *J* = 12.0 Hz, C6), 1.30-1.22 (m, 8H, C42-C45), 1.17 (s, 3H, -CH_3), 1.01 (bs, 12H, -CH_3, -TBDPS), 0.95 (s, 3H, -CH_3), 0.87 (t, 3H, *J* = 7.0 Hz, C46), 0.83 (s, 3H, -CH_3), 0.81 (s, 9H, -TBS), -0.09 (s, 3H, -TBS), -0.10 (s, 3H, -TBS) ppm.

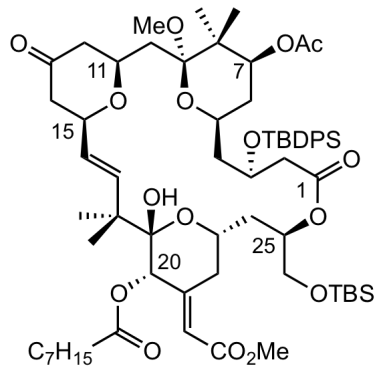
^{13}C NMR ($CDCl_3$, 125 MHz,): δ = 207.4, 172.2, 170.9, 170.3, 166.9, 151.3, 136.1, 135.9, 135.8, 134.8, 133.8, 132.3, 130.1, 130.0, 127.9, 127.8, 120.1, 102.6, 98.1, 76.7, 74.1, 73.9, 71.9, 69.8, 67.1, 65.4, 65.0, 64.6, 51.4, 48.6, 47.9, 47.8, 45.1, 44.9, 42.6, 41.7, 39.4, 37.1, 34.7, 33.5, 31.8, 31.1, 29.1, 29.0, 27.1, 25.9, 24.8, 23.9, 22.7, 21.5, 20.4, 20.2, 19.4, 18.3, 17.4, 14.2, -5.2, -5.3 ppm.

IR: 3519, 2954, 2931, 2858, 1731, 1665, 1470, 1430, 1386, 1365, 1247, 1154, 1106, 1023, 1004, 986, 838, 778, 740, 705 cm^{-1}

HRMS (TOF MS ES+): Calculated for $C_{66}H_{100}O_{16}Si_2Na^+$: 1227.6442; Found: 1227.6492.

$[\alpha]_D^{22.2}$ = + 20.2 ± 0.6 º (*c* = 0.45, CH_2Cl_2)

$\mathbf{R}_f$ = 0.57 (20% EtOAc in petroleum ether); one brown spot, *p*-anisaldehyde stain.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O
O
15
OTBDPS
O
1
OH
O
O
25
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OTBS
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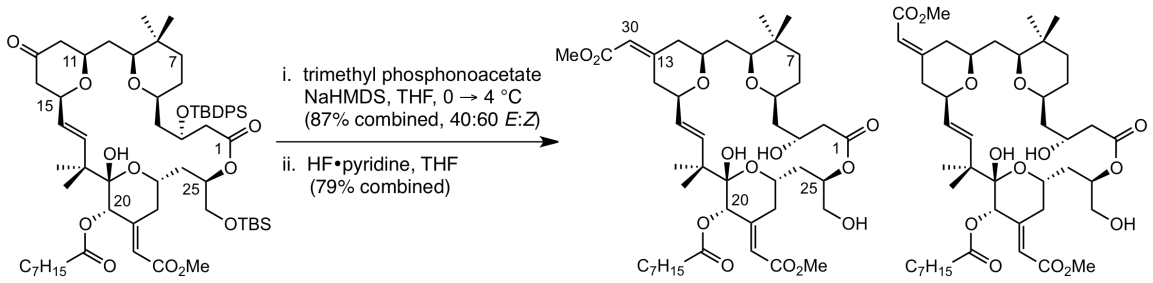
O
CO_2Me

25

8
7
6
5
4
3
2
1
0
ppm



Procedure for analog 5 and its C13-C30 geometric isomer S5

A solution of trimethyl phosphonoacetate (155 μL, 0.958 mmol) in THF (8.3 mL) was cooled in an ice water bath under N_2 atmosphere. A solution of NaHMDS (1.0 M in THF, 0.85 mL, 0.85 mmol) was then added dropwise over 30 sec. After 30 min, an aliquot (2.04 mL, 0.210 mmol trimethyl phosphonoacetate) of the resulting cloudy, white mixture was added to neat ketone **23** (8.9 mg, 0.0080 mmol), previously cooled in an ice water bath under N_2 atmosphere. The resulting mixture was stirred 20 h, at which time H_2O (3 mL) and Et_2O (5 mL) was added. The layers were separated, and the aqueous layer was extracted with Et_2O (3 x 5 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The resulting oil was purified via silica gel chromatography (10→12→13% EtOAc:Pentane) to afford 8.1 mg **26** (87%) as a 1.0:1.5 *E*:*Z* mixture of enoate isomers, in agreement with crude ^{1}H NMR selectivity analysis. The crude mixture was used directly in the next step.

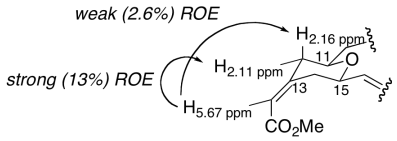
In a 15 mL polypropylene vial, the above enoate mixture (8.1 mg, ca 0.0069 mmol) was dissolved in THF (6.2 mL) under N_2 atmosphere. This solution was cooled in a CO_2/acetone bath for 10 min, at which time 70% HF•pyridine (1.55 mL) was added dropwise over 1 min. The reaction vessel was allowed to stir at -78 °C for 10 more min, at which time the reaction vessel was removed from the cold bath and allowed to warm to ambient temperature. After stirring 90 h, the reaction mixture was quenched into saturated aqueous $NaHCO_3$ (50 mL). The resulting mixture was diluted with water (15 mL) and Et_2O (30 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated. The resulting oil was purified via silica gel column chromatography (45→50% EtOAc:Pentane) to afford 4.5 mg (79%) as a mixture of enoate analogues **5** (*Z*-enoate) and C13-C30 geometric isomer **S5** (*E*-enoate). This mixture was separated via preparative reverse-phase C18 chromatography (65% MeCN→100% MeCN in H_2O over 30 minutes) to afford 2.2 mg analogue **5** (38%) and 1.1 mg C13-C30 geometric isomer **S5** (19%).

Characterization data for analog **5**:

^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.99 (d, *J* = 1.7 Hz, 1H, C27), 5.74 (d, *J* = 15.9 Hz, 1H, C17), 5.67 (s, 1H, C30), 5.35 (m, 1H, C25), 5.33 (dd, *J* = 8.3, 15.9 Hz, 1H, C16), 5.18 (s, 1H, C19-OH), 5.13 (s, 1H, C20), 4.96 (d, *J* = 11.7 Hz, 1H, C3-OH), 4.22 (m, 1H, C3), 4.06 (tt, *J* = 2.2, 11.2 Hz, 1H, C23), 3.97 (ddd, *J* = 2.2, 8.3, 10.7 Hz, 1H, C15), 3.84 (dt, *J* = 3.9, 12.0 Hz, 1H, C26), 3.68-3.75 (m, 1H, C22), 3.70 (s, 3H, -OMe), 3.68 (s, 3H, -OMe), 3.60-3.66 (m, 2H, C14, C26), 3.55 (ddd, *J* = 2.7, 6.4, 10.7 Hz, 1H, C11), 3.49 (bt, *J* = 11.2 Hz, 1H, C5), 3.13 (dd, *J* = 1.7, 11.5 Hz, 1H, C9), 2.40-2.49 (m, 2H, C2, C2), 2.25-2.35 (m,

2H, C31), 2.08-2.23 (m, 2H, C12, C12), 1.92-2.08 (m, 4H, C4, C22, C24, C26-OH), 1.89 (bt, J = 12.7 Hz, 1H, C14), 1.79 (ddd, J = 2.7, 11.7, 13.7 Hz, 1H, C24), 1.70 (ddd, J = 6.6, 11.7, 15.4 Hz, 1H, C10), 1.62 (m, 2H, C32), 1.41-1.53 (m, 3H, C4, C6, C10), 1.21-1.38 (m, 11H, C6, C7, C7, C33-C36), 1.15 (s, 3H, C28), 1.01 (s, 3H, C29), 0.88 (t, J = 6.8 Hz, 3H, C37), 0.86 (s, 3H, C(8)(CH_3)$_2$), 0.81 (s, 3H, C(8)(CH_3)), ppm.

ROESY ($CDCl_3$, 600 MHz)* Assignment of enoate geometry:



*ROESY enhancements reported relative to geminal C-H ROE crosspeak volumes set at 100%

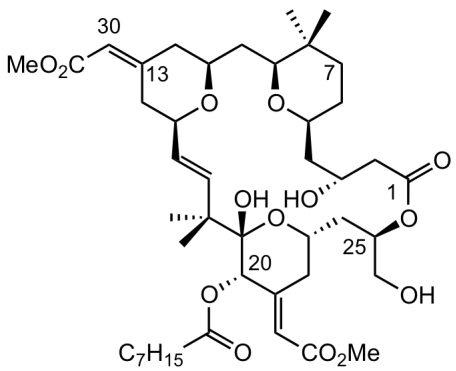
^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.5, 172.3, 167.2, 166.9, 157.0, 152.0, 138.6, 130.0, 119.9, 114.4, 99.0, 86.2, 79.0, 76.2, 74.4, 71.6, 68.8, 65.9, 64.6, 51.2, 51.2, 45.0, 44.2, 43.0, 40.3, 39.0, 37.0, 36.6, 36.0, 34.8, 32.4, 31.8, 31.2, 29.2, 29.0, 29.0, 27.8, 24.8, 24.7, 22.7, 19.9, 19.4, 14.2 ppm.

IR (film) 3458, 3338, 2930, 2851, 1721, 1658, 1434, 1407, 1378, 1282, 1258, 1226, 1160, 1099, 1084, 1046, 1004, 877, 807 cm^{-1}.

HRMS (TOF MS ES+): Calculated for $C_{44}H_{68}O_{14}Na^+$: 843.4507; Found: 843.4501.

$[\alpha]_D^{23.7}$ = 21.47° (c = 0.22, $CHCl_3$).

R_f = 0.44 (60%% EtOAc , 40% Petroleum ether) – one black spot (*p*-anisaldehyde stain).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O
7
O
1
OH
HO
O
O
25
20
OH
O
C7H15
O
CO2Me

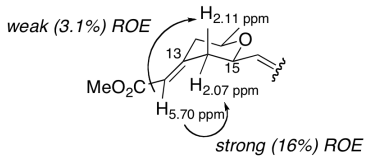
5

11
10
9
8
7
6
5
4
3
2
1
-0
-1
ppm

Characterization data for *E*-C13-C30 isomer **S5**:

^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.99 (d, *J* = 1.7 Hz, 1H, C27), 5.75 (d, *J* = 11.5 Hz, 1H, C17), 5.70 (s, 1H, C30), 5.36 (m, 1H, C25), 5.32 (dd, *J* = 8.3, 15.9 Hz, 1H, C16), 5.18 (s, 1H, C19-OH), 5.14 (s, 1H, C20), 4.49 (d, *J* = 11.7 Hz, 1H, C3-OH), 4.21 (m, 1H, C3), 3.96-4.12 (m, 2H, C15, C23), 3.84 (bt, *J* = 3.7, 12.2 Hz, 1H, C26), 3.79 (d, *J* = 13.7 Hz, 1H, C12), 3.69-3.74 (m, 1H, C22), 3.69 (s, 3H, -OMe), 3.68 (s, 3H, -OMe), 3.64 (m, 1H, C26), 3.42-3.53 (m, 2H, C5, C11), 3.15 (dd, *J* = 2.0, 11.5 Hz, 1H, C9), 2.39-2.55 (m, 2H, C2, C2), 2.25=2.37 (m, 2H, C31), 1.97-2.19 (m, 5H, C4, C14, C14, C24, C26-OH), 1.85-1.97 (m, 2H, C12, C22), 1.79 (ddd, *J* = 2.7, 11.7, 13.7 Hz, 1H, C24), 1.70 (ddd, *J* = 6.7, 12.0, 15.4 Hz, 1H, C10), 1.56-1.69 (m, 3H, C6, C32, C32), 1.38-1.52 (m, 2H, C4, C10), 1.18-1.38 (m, 11H, C6, C7, C7, C33-36), 1.14 (s, 3H, C28), 1.01 (s, 3H, C29), 0.87 (t, *J* = 6.8 Hz, 3H, C37), 0.86 (s, 3H, C(8)$(CH_3)_2$), 0.81 (s, 3H, C(8)$(CH_3)_2$) ppm.

ROESY ($CDCl_3$, 600 MHz)* Assignment of enoate geometry:



*ROESY enhancements reported relative to geminal C-H ROE crosspeak volumes set at 100%

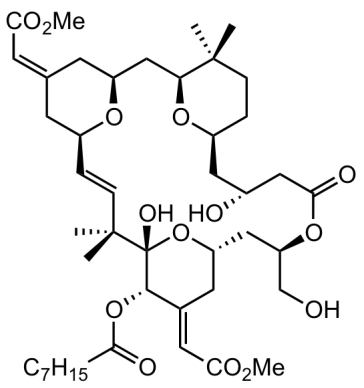
^{13}C NMR ($CDCl_3$, 125 MHz): δ 173.6, 172.3, 167.3, 167.2, 157.5, 151.8, 139.0, 129.7, 120.0, 114.2, 99.0, 86.2, 79.7, 76.2, 74.4, 71.5, 68.8, 65.8, 64.7, 51.2, 51.1, 45.0, 43.3, 42.9, 40.4, 39.0, 37.5, 37.1, 36.0, 34.8, 32.4, 31.8, 31.2, 29.9, 29.2, 29.1, 29.0, 27.8, 24.8, 24.7, 22.7, 19.8, 19.4, 14.2 ppm.

IR (film) 3455, 3338, 2929, 2851, 1720, 1658, 1435, 1407, 1377, 1363, 1324, 1280, 1249, 1230, 1206, 1152, 1098, 1085, 1048, 1027, 1004, 985, 885, 804, 756, 699, 665 cm^{-1}.

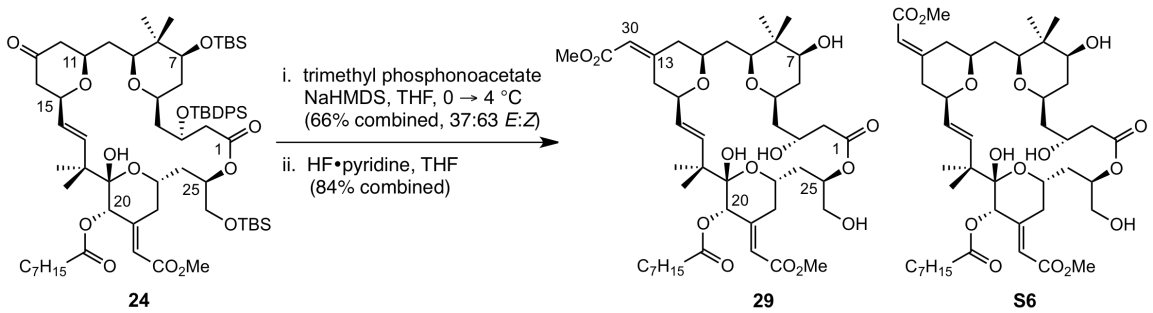
HRMS (TOF MS ES+): Calculated for $C_{44}H_{68}O_{14}Na^{+}$: 843.4507; Found: 843.4496.

$[\alpha]_D^{23.9}$ = -27.82° (*c* = 0.11, $CHCl_3$).

$\mathbf{R}_f$ = 0.48 (60% EtOAc , 40% Petroleum ether) – one black spot (*p*-anisaldehyde stain).



S5



Procedure for enoate 29 and its C13-C30 geometric isomer S6

Trimethyl phosphonoacetate (0.155 mL, 0.958 mmol, Aldrich) was dissolved in THF (8.3 mL) in a flame-dried round bottom flask under positive pressure of N_2 charged with a Teflon-coated magnetic stir bar. The solution was cooled to 0 °C in an ice bath (temperature monitored externally), and NaHMDS (0.85 mL, 1.0 M in THF, 0.85 mmol, Aldrich) was added dropwise via syringe over 20 s. The solution was stirred at 0 °C for 20 min, at which point an aliquot (4.5 mL, 0.48 mmol) of the cloudy white solution was added dropwise via syringe over 10 s to C13 ketone-containing bryopyran **24** (11.4 mg, 0.009 mmol), which had been cooled to 0 °C in a vial that was under positive pressure of N_2 and charged with a Teflon-coated magnetic stir bar. After 5 h, starting material remained by TLC, so the reaction vial was placed in the freezer at -20 °C overnight. After 17 h of total reaction time, the reaction was quenched with saturated NH_4Cl (5 mL) and diluted with H_2O (2.5 mL) and Et_2O (10 mL). The organic layer was separated, and the aqueous layer was washed with Et_2O (3 x 10 mL). The combined organic layers were dried with $MgSO_4$, filtered, and reduced. Flash chromatography on silica (10% EtOAc: Pentane) provided 7.9 mg (66%) of product as a colorless oil (1.7 : 1 mixture of *Z*:*E* olefin isomers by ^{1}H-NMR analysis).

This oil mixture from the previous step (7.9 mg, 0.006) was dissolved in THF (5.5 mL) in a polypropylene vial under positive pressure of N_2 and charged with a Teflon-coated magnetic stir bar. The solution was cooled to –78 °C in a CO_2/acetone bath, at which point HF•pyridine (1.4 mL, 70% HF, 30% pyridine, Aldrich) was added dropwise via syringe over 30 s. After 20 min, the reaction was allowed to warm to room temperature. After 24 h, the reaction was transferred in three portions into saturated $NaHCO_3$ (40 mL) and diluted with H_2O (8 mL) and EtOAc (30 mL). The organic layer was separated, and the aqueous layer was washed with EtOAc (3 x 40 mL). Flash chromatography on silica (95 % EtOAc: Pentane) provided 4.3 mg (84% combined) of a mixture of enoate isomers **29** and **S6** as a colorless oil. The mixture was purified via repeated analytical HPLC (50%-95% MeCN in H_2O, 30 min, 1 mg loadings) purifications to provide 2.5 mg pure **29** and 1.2 mg pure **S6** as white solids.

Characterization data for enoate **29**:

^{1}H-NMR (600 MHz, $CDCl_3$): δ 5.99 (1H, s, C27), 5.73 (1H, d, *J* = 15.8 Hz, C17), 5.68 (1H, s, C38), 5.38-5.29 (2H, m, C16, C25), 5.14 (1H, s, C20), 5.13 (1H, s, C19OH), 4.39 (1H, d, *J* = 11.8 Hz, C3OH), 4.27-4.20 (1H, m, C3), 4.08-4.02 (1H, m, C23), 4.00-3.95 (1H, m, C15), 3.86-3.81 (1H, d, *J* = 11.8 Hz, C26), 3.74-3.66 (1H, m, C22), 3.70 (3H, s, -OMe), 3.68 (3H, s, -OMe), 3.66-3.57 (3H, m, C5, C14, C26), 3.57-3.52 (1H, m, C11), 3.37 (1H, d, *J* = 11.5 Hz, C7), 3.08 (1H, d, *J* = 11.4 Hz, C9), 2.52-2.37 (2H, m, C2),

2.37-2.25 (2H, m, C31), 2.22-1.95 (5H, m, C12, C22, C24, C7OH, C26OH), 1.95-1.86 (3H, m, C12, C14, C24), 1.85-1.74 (2H, m, C32), 1.67-1.49 (2H, m, C10, C4), 1.43 (1H, q, J = 11.8 Hz, C10), 1.41-1.35 (1H, m, C4), 1.33-1.20 (10H, m, C33, C34, C35, C36, C6), 1.15 (3H, s, -C(CH_3)$_2$), 1.01 (3H, s, -C(CH_3)$_2$), 0.92 (3H, s, -C(CH_3)$_2$), 0.88 (3H, t, J = 6.7 Hz, C37), 0.81 (3H, s, -C(CH_3)$_2$) ppm.

^{13}C-NMR (125 MHz, C_6D_6): δ 172.32, 172.22, 167.2, 166.9, 156.7, 151.9, 138.7, 129.9, 119.9, 114.5, 99.0, 84.9, 75.0, 74.4, 74.0, 71.6, 68.6, 65.8, 64.7, 51.24, 51.18, 45.0, 44.1, 42.9, 40.04, 40.02, 39.0, 37.3, 36.5, 36.3, 35.9, 34.8, 31.8, 31.2, 29.8, 29.16, 29.03, 24.83, 24.68, 22.72, 22.57, 19.9, 14.2, 12.6 ppm.

ROESY (600MHz, C_6D_6): indicates that the C13 enoate in **29** has the indicated *Z* geometry. ROESY correlations are observed between the C38 (5.68, s) enoate proton and the C14 protons (3.66-3.57, m, 1.95-1.86, m) on the B ring.

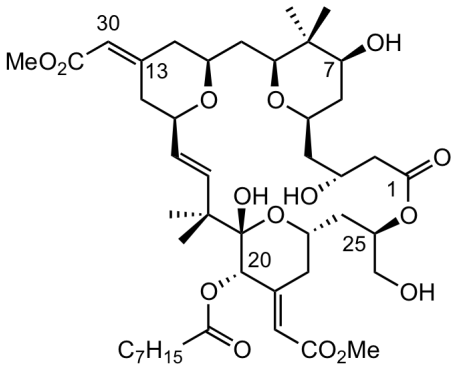
IR (**film**): 3449, 2929, 2855, 1719, 1654, 1436, 1406, 1378, 1363, 1259, 1227, 1161, 1085, 1047, 1027, 1003, 985, 867, 801 cm^{-1}.

HRMS (TOF MS ES$^+$): calculated for $C_{44}H_{68}O_{15}Na$: 859.4456; Found: 859.4468.

$[\alpha]_D^{22.8}$ = 7.5 ^{o} (c = 0.6, CH_2Cl_2).

R_f = 0.29 (95% EtOAc: pentane) – one black spot, *p*-anisaldehyde stain.

HPLC: Retention Time = 16.87 min. Method: Analytical column; 65% - 95% MeCN in H_2O, 30 min).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O
O
25
20
OH
O
C7H15
O
CO2Me

29

ppm 9
8
7
6
5
4
3
2
1
0

Characterization data for *E*-C13-C30 isomer **S6**:

^{1}H-NMR (600 MHz, $CDCl_3$): δ 5.99 (1H, s, C27), 5.75 (1H, d, *J* = 15.9 Hz, C17), 5.71 (1H, s, C38), 5.39-5.34 (1H, m, C25), 5.32 (1H, dd, *J* = 8.4, 15.8 Hz, C16), 5.14 (1H, s, C20), 5.14 (1H, s, C19OH), 4.42 (1H, d, *J* = 11.8 Hz, C3OH), 4.26-4.20 (1H, m, C3), 4.09-4.01 (2H, m, C15, C23), 3.86-3.78 (2H, m, C12, C26), 3.75-3.66 (1H, m, C22), 3.69 (3H, s, -OMe), 3.68 (3H, s, -OMe), 3.66-3.57 (2H, m, C5, C26), 3.52-3.46 (1H, m, C11), 3.37 (1H, d, *J* = 11.3 Hz, C7), 3.09 (1H, d, *J* = 11.6 Hz, C9), 2.50-2.38 (2H, m, C2), 2.37-2.24 (2H, m, C31), 2.17-1.96 (6H, m, C14, C22, C24, C7OH, C26OH), 1.95-1.87 (2H, m, C4, C12), 1.85-1.75 (2H, m, C10, C24), 1.68-1.47 (4H, m, C6, C10, C32), 1.47-1.34 (2H, m, C4, C6), 1.35-1.19 (8H, m, C33, C34, C35, C36), 1.14 (3H, s, $-C(CH_3)_2$), 1.01 (3H, s, $-C(CH_3)_2$), 0.92 (3H, s, $-C(CH_3)_2$), 0.87 (3H, t, *J* = 6.5 Hz, C37), 0.82 (3H, s, $-C(CH_3)_2$) ppm.

^{13}C-NMR (125 MHz, $CDCl_3$): δ 172.34, 172.27, 167.28, 167.13, 157.3, 151.8, 139.0, 129.6, 120.0, 114.3, 99.0, 84.9, 79.7, 75.0, 74.4, 74.0, 71.6, 68.7, 65.8, 64.7, 51.26, 51.13, 45.0, 43.3, 42.9, 40.1, 39.0, 37.42, 37.31, 36.4, 35.9, 34.8, 31.8, 31.1, 29.9, 29.16, 29.03, 24.83, 24.70, 22.72, 22.57, 19.8, 14.2, 12.7 ppm.

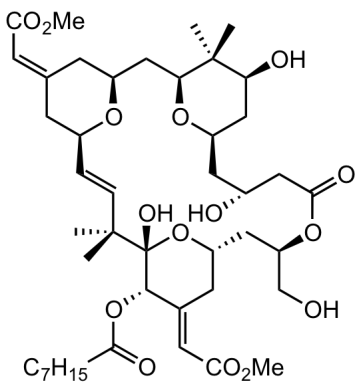
IR (**film**): 3454, 2928, 2855, 1719, 1655, 1435, 1407, 1377, 1260, 1229, 1152, 1085, 1048, 1004, 986, 859, 804 cm^{-1}.

HRMS (TOF MS ES^+): calculated for $C_{44}H_{68}O_{15}Na$: 859.4456; Found: 859.4450.

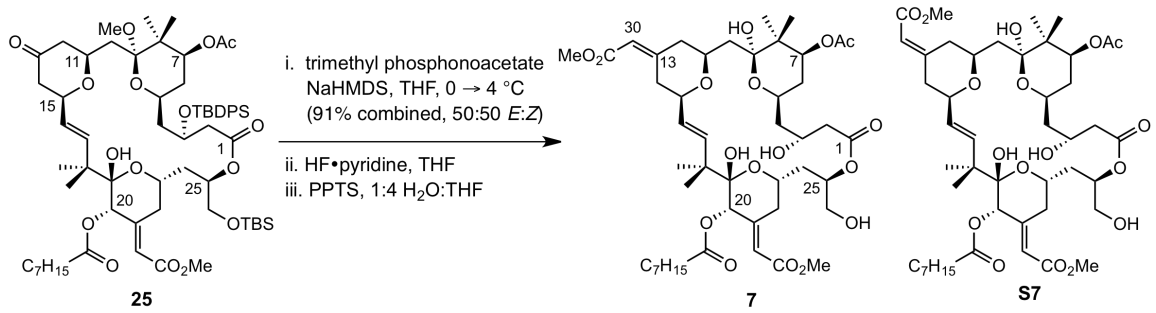
$[\alpha]_D^{22.8}$ = -54.9 ^{o} (c = 0.2, CH_2Cl_2).

$\mathbf{R}_f$ = 0.24 (95% EtOAc: pentane) – one black spot, *p*-anisaldehyde stain.

HPLC: Retention Time = 18.44 min. Method: Analytical column; 65% - 95% MeCN in H_2O, 30 min).



S6



Procedure for analog 7 and its C13-C30 geometric isomer S7

A solution of timethyl phosphonoacetate (155 μL, 0.96 mmol) in THF (8.3 mL) was cooled to 0 ºC in an ice water bath. NaHMDS (1.0 M in THF, 0.85 mL, 0.85 mmol) was added dropwise over 30 s by syringe, and the resulting mixture was stirred at 0 ºC for 30 min, by which time a white suspension had formed. This mixture (ca. 0.09 M in sodium phosphonate reagent) was used immediately in the following step.

Neat ketone **25** (6.0 mg, 0.0050 mmol) was cooled to 0 ºC in an ice water bath. An aliquot of the aforementioned sodium phosphonate reagent (ca. 0.09 M in THF, 550 μL, 0.05 mmol) was added in one portion by syringe, and the resulting mixture was stirred at 0 ºC. After 4.75 h at 0 ºC, the reaction was moved to a cold room at 4 ºC where stirring was continued for an additional 18 h. The reaction was then quenched with saturated aqueous NH_4Cl (1.5 mL) and the biphasic mixture was diluted with Et_2O (1 mL). The phases were separated, and the aqueous phase was extracted with Et_2O (4 x 1 mL). The combined organic phase was dried over $MgSO_4$, filtered, and concentrated to afford a crude residue that was partially purified by silica gel chromatography (16% EtOAc in hexanes) to afford 5.7 mg of a ~1:1 mixture of C13-C30 enoate isomers (91% combined yield).

In a polypropylene vial, the aforementioned enoate mixture (ca. 0.0045 mmol) was dissolved in THF (2.0 mL). The resulting solution was cooled in an ice water bath and HF·pyridine (70% HF, 500 μL) was added dropwise over 30 s. The mixture was stirred at 0 ºC for 5 min and was then warmed to ambient temperature. After 74 h, the reaction was quenched by its dropwise addition to stirred, saturated aqueous $NaHCO_3$ (20 mL). This mixture was extracted with EtOAc (3 x 20 mL) and the combined organic phase was washed with 30 mL 0.2 N HCl. The acidic aqueous phase was extracted with additional EtOAc (2 x 20 mL). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated to afford a crude residue that was used immediately in the following step.

The crude residue from the previous step was dissolved in a solution of PPTS (0.05 M in 20% H_2O:THF, 600 μL, 0.03 mmol) at ambient temperature. After 39 h, the reaction was then diluted with Et_2O (500 μL), H_2O (500 μL), and brine (500 μL). The organic an aqueous phases were separated, and the aqueous phase was extracted with Et_2O (5 x 1 mL). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated to afford a crude residue that was partially purified by flash chromatography (60% EtOAc in hexanes) to afford a mixture of enoate analogues that was separated by reverse phase HPLC (C18, 10 micron, 10 x 250 mm, 65 → 100 % MeCN in H_2O) to afford 1.3 mg Z-enoate analogue **7** (29% over 3 steps) and 1.2 mg E-enoate analogue **S7** (27% over 3 steps) as amorphous white solids. Compound purity was established by TLC (one spot) and ^{1}H-NMR analysis.

Characterization data for analog **7**:

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 5.98 (d, 1H, *J* = 1.8 Hz, C34), 5.74 (d, 1H, *J* = 15.7 Hz, C17), 5.68 (bs, 1H, C30), 5.32 (m, 1H, C25), 5.32 (dd, 1H, *J* = 8.4, 15.7 Hz), 5.16 (s, 1H, C19-OH), 5.14 (m, 1H, C7), 5.13 (s, 1H, C20), 4.24 (d, 1H, *J* = 12.0 Hz, C3-OH), 4.23 (m, 1H, C5), 4.17 (m, 1H, C3), 4.04 (tt, 1H, *J* = 2.2, 11.3 Hz, C23), 3.98 (ddd, 1H, *J* = 2.3, 8.6, 11.1 Hz, C15), 3.84 (m, 1H, C26), 3.78 (m, 1H, C13), 3.72-3.61 (m, 3H, C14, C22, C26), 3.70 (s, 3H, -CO_2Me), 3.68 (s, 3H, -CO_2Me), 2.52-2.42 (m, 3H, C2, C2, C19-OH), 2.31 (m, 2H, C40, C40), 2.21 (m, 1H, C12), 2.14-1.96 (m, 6H, C4, C10, C12, C22, C24, C26-OH), 2.05 (s, 3H, C7-OAc), 1.91 (m, 1H, C14), 1.78 (m, 2H, C6, C24), 1.67 (d, 1H, *J* = 15.4 Hz, C10), 1.64-1.53 (m, 3H, C4, C41, C41), 1.48 (q, 1H, *J* = 12.0 Hz, C6), 1.27 (m, 8H, C42-C45), 1.15 (s, 3H, -CH_3), 1.00 (s, 6H, -CH_3, -CH_3), 0.95 (s, 3H, -CH_3), 0.87 (t, 3H, *J* = 7.1 Hz, C46) ppm.

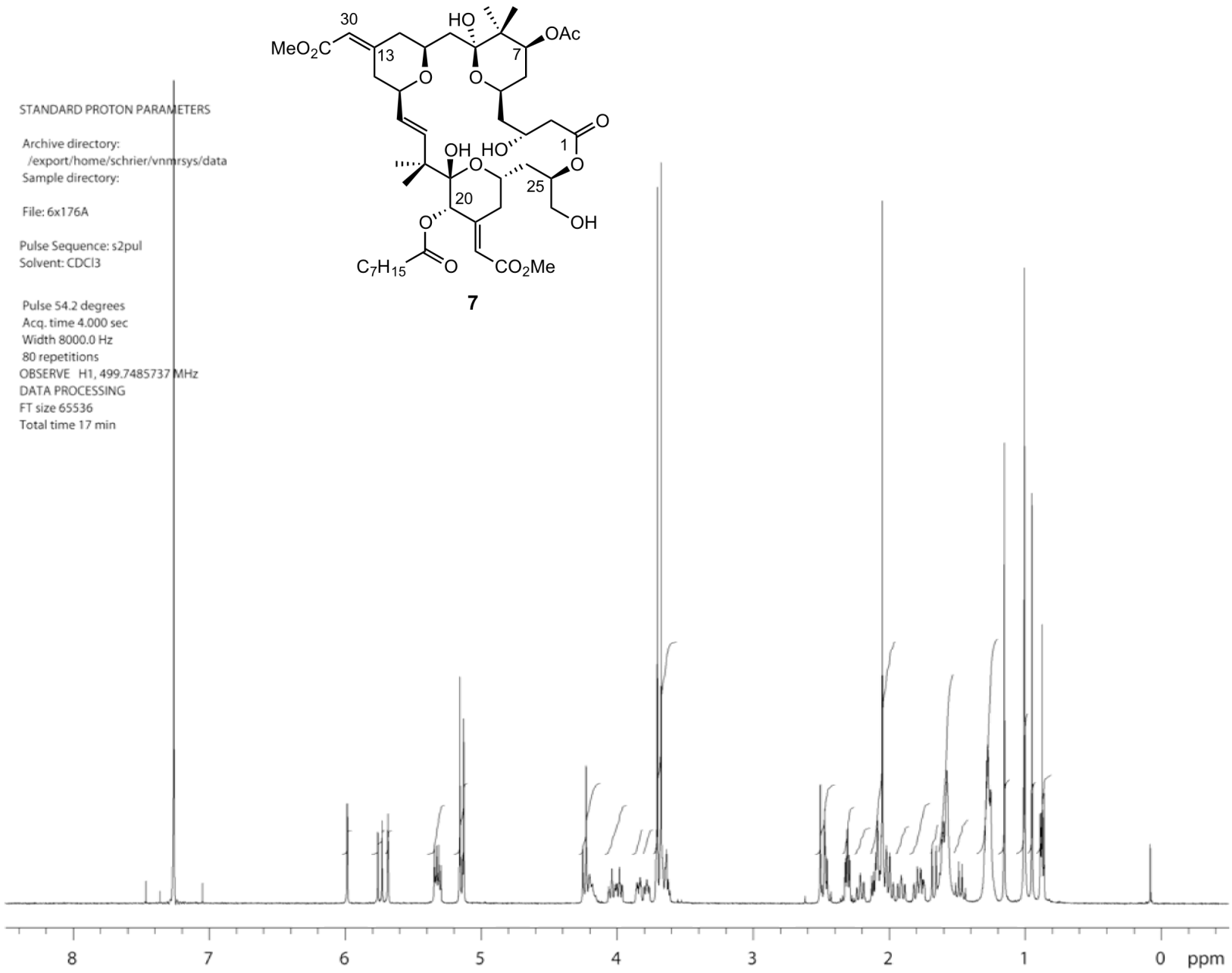
^{13}C NMR ($CDCl_3$, 100 MHz): δ = 172.5, 172.4, 171.1, 167.1, 166.8, 156.5, 151.9, 139.1, 129.7, 119.9, 114.6, 102.0, 99.0, 79.3, 74.4, 73.0, 71.7, 71.6, 68.5, 65.9, 65.6, 64.8, 51.3, 51.2, 45.0, 44.2, 42.4, 42.1, 41.1, 39.8, 36.4, 35.9, 34.8, 33.4, 31.8, 31.2, 29.2, 29.0, 24.8, 24.7, 22.7, 21.3, 21.2, 19.9, 17.0, 14.2 ppm.

IR: 3466, 3350, 2930, 1738, 1722, 1715, 1660, 1435, 1408, 1366, 1247, 1157, 1079, 1030, 1003, 984, 859, 811 cm^{-1}

HRMS (TOF MS ES+): Calculated for $C_{46}H_{70}O_{17}Na^{+}$: 917.4505; Found: 917.4523.

$[\alpha]_D^{23.2}$ = + 42.6 ± 1.1 ° (*c* = 0.23, CH_2Cl_2)

$\mathbf{R}_f$ = 0.29 (50% EtOAc in petroleum ether), one purple spot, *p*-anisaldehdye stain.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DATA PROCESSING
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Total time 17 min
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OAc
MeO2C
13
7
O
O
OH
HO
1
O
O
20
25
OH
O
C7H15
O
CO2Me
8
7
6
5
4
3
2
1
0
ppm

7

Characterization data for *E*-C13-C30 isomer **S7:**

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 5.98 (d, 1H, *J* = 1.7 Hz, C34), 5.76 (d, 1H, *J* = 15.8 Hz, C17), 5.71 (bs, 1H, C30), 5.32 (m, 1H, C25), 5.30 (dd, 1H, *J* = 8.5, 15.8 Hz, C16), 5.17 (s, 1H, C19-OH), 5.15 (m, 1H, C7), 5.13 (s, 1H, C20), 4.27 (d, 1H, *J* = 12.0 Hz, C3-OH), 4.25 (m, 1H, C5), 4.18 (m, 1H, C3), 4.08-4.00 (m, 2H, C15, C23), 3.83 (m, 1H, C26), 3.75 (m, 1H, C11), 3.72-3.60 (m, 3H, C12, C22, C26), 3.69 (s, 3H, -CO_2Me), 3.68 (s, 3H, -CO_2Me), 2.75 (s, 1H, C9-OH), 2.50-2.44 (m, 2H, C2, C2), 2.34-2.24 (m, 2H, C40, C40), 2.17-2.05 (m, 4H, C10, C14, C14, C26-OH), 2.05 (s, 3H, C7-OAc), 2.06-1.90 (m, 4H, C4, C12, C22, C24), 1.82-1.73 (m, 3H, C6, C10, C24), 1.67-1.55 (m, 3H, C4, C41, C41), (*app*-q, 1H, *J* = 12.0 Hz, C6), 1.33-1.20 (m, 8H, C42-C45), 1.14 (s, 3H, -CH_3), 1.00 (s, 6H, -CH_3, -CH_3), 0.95 (s, 3H, -CH_3), 0.87 (t, 3H, *J* = 7.1 Hz, C46) ppm.

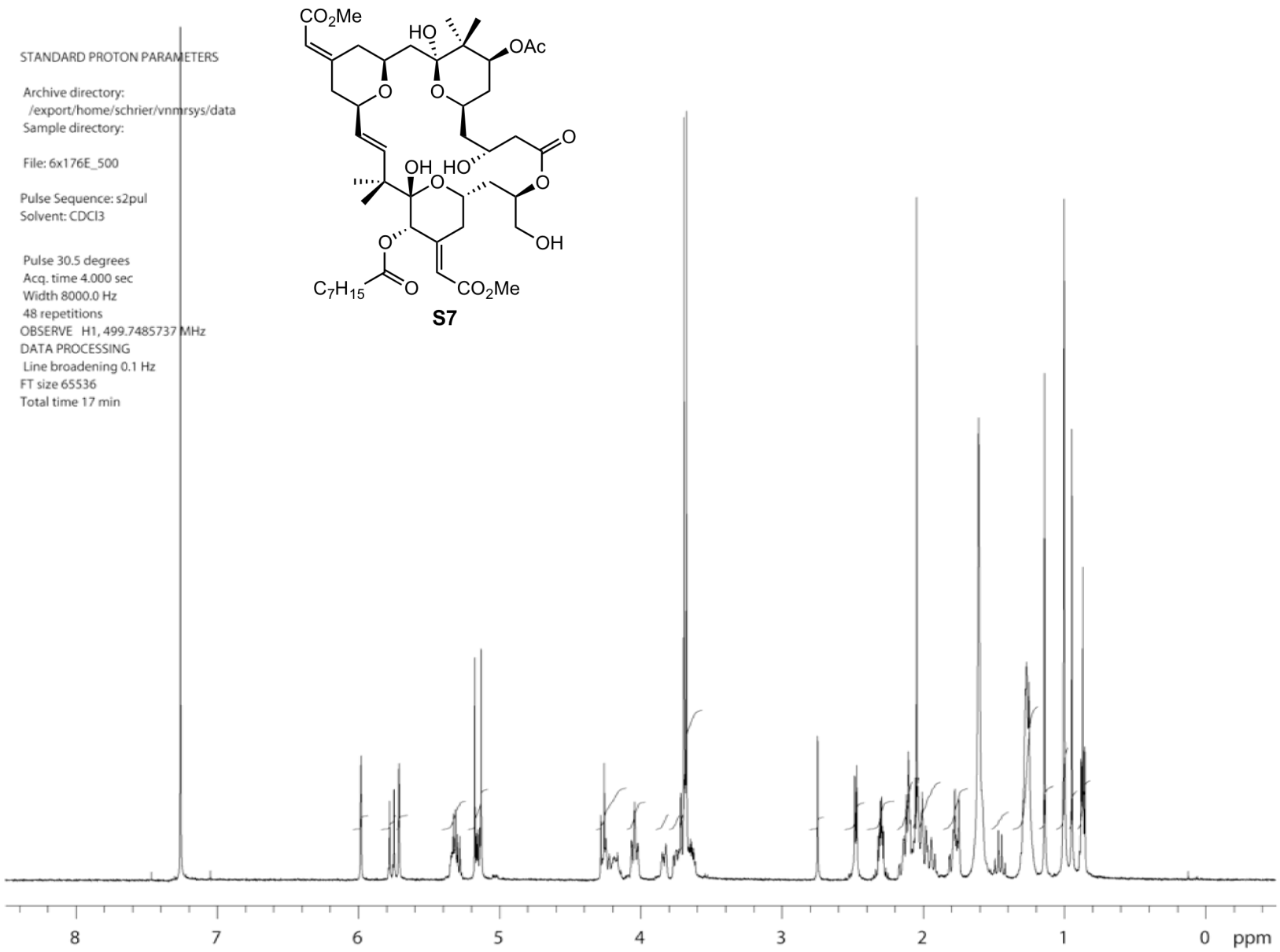
^{13}C NMR ($CDCl_3$, 125 MHz): δ = 172.6, 172.3, 171.0, 167.2, 167.1, 156.8, 151.7, 139.4, 129.3, 120.0, 114.5, 101.9, 99.0, 80.0, 74.4, 73.0, 71.6, 71.6, 68.6, 65.9, 65.6, 64.8, 51.3, 51.2, 45.0, 43.1, 42.4, 42.2, 41.2, 40.0, 37.6, 36.0, 34.8, 33.5, 31.8, 31.1, 29.2, 29.0, 24.8, 24.7, 22.7, 21.3, 21.2, 19.8, 17.0, 14.2 ppm.

IR: 3466, 3349, 2931, 1722, 1659, 1435, 1408, 1366, 1235, 1155, 1078, 1028, 1003, 985, 855, 812 cm^{-1}

HRMS (TOF MS ES+): Calculated for $C_{46}H_{70}O_{17}Na^{+}$: 917.4505; Found: 917.4523.

$[\alpha]_D^{22.3}$ = – 38.5 ± 1.0 ° (*c* = 0.11, MeOH).

$\mathbf{R}_f$ = 0.38 (50% EtOAc in petroleum ether), one purple spot, *p*-anisaldehdye stain.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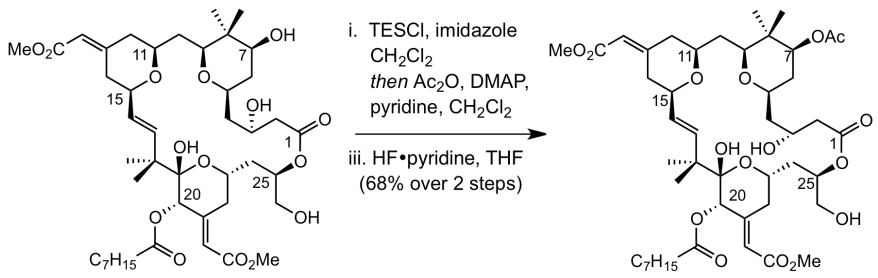


29 **6**

Procedure for analog 6

Ester **29** (3.6 mg, 0.004 mmol) was dissolved in CH_2Cl_2 (0.1 mL) in an oven-dried, Teflon-capped vial charged with a Teflon-coated magnetic stir bar and purged with N_2. DMAP (1.0 mg, 0.008 mmol, Aldrich) was then added in one portion, and the solution was cooled to –10 °C in an ice/acetone bath (temperature monitored externally). TESCl (2.4 μL, 0.007 mmol, Aldrich) was then added in one portion via microsyringe. After 15 min, the vial was allowed to warm to room temperature. After an additional 20 min, the reaction was quenched with saturated NH_4Cl (0.3 mL) and diluted with H_2O (0.5 mL) and Et_2O (1 mL). The organic layer was separated, and the aqueous layer was washed with Et_2O (4 x 1 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated to afford a pale yellow oil.

The crude, C26 TES protected product (4.1 mg, 0.004 mmol) from above was dissolved in CH_2Cl_2 (0.1 mL) in a Teflon-capped, oven-dried vial purged with N_2 and charged with a Teflon-coated magnetic stir bar. Pyridine (0.5 μL, 0.006 mmol, Aldrich) was added in one portion via microsyringe, followed by the addition of DMAP (0.6 mg, 0.005 mmol, Aldrich) in one portion. Next, Ac_2O (0.6 μL, 0.002 mmol, Aldrich) was added in one portion via microsyringe. The reaction was stirred at room temperature for 2 h, at which point it was quenched with saturated NH_4Cl (0.3 mL) and diluted with H_2O (0.2 mL) and Et_2O (1 mL). The organic layer was separated, and the aqueous layer was washed with Et_2O (4 x 1 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated to afford a crude oil.

The crude acylated product from above (4.3 mg, 0.004 mmol) was dissolved in THF (4.1 mL) in a polypropylene vial equipped with a Teflon-coated magnetic spin vane and under positive pressure of N_2 gas. The solution was cooled to –78 °C in a CO_2/acetone bath, and HF•pyridine (925 μL, 70% HF, 30% pyridine, Aldrich) was added dropwise via syringe over 30 s. After 20 min, the reaction was allowed to warm to room temperature. After 12 h, the reaction was transferred in three portions to saturated $NaHCO_3$ (25 mL) and diluted with H_2O (5 mL) and EtOAc (10 mL). The organic layer was separated, and the aqueous layer was washed with EtOAc (3 x 25 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated *in vacuo*. Flash chromatography on silica (60% EtOAc: Pentane) provided 2.6 mg (68%, two steps) of **6** as a white solid.

Characterization data for analog **6**:

^{1}H-NMR (600 MHz, $CDCl_3$): δ 5.98 (1H, d, *J* = 1.6 Hz, C27), 5.74 (1H, d, *J* = 15.8 Hz, C17), 5.68 (1H, s, C38), 5.38-5.33 (1H, m, C25), 5.33 (1H, dd, *J* = 8.0, 15.7 Hz, C16), 5.14 (1H, s, C20), 5.13 (1H, s, C19OH), 4.57 (1H, dd, *J* = 4.6, 11.6 Hz, C7), 4.36 (1H, d, *J* = 11.8 Hz, C3OH), 4.27-4.20 (1H, m, C3),

4.08-4.01 (1H, tt, *J* = 2.2, 11.3 Hz, C23), 4.01-3.96 (1H, ddd, *J* = 2.3, 8.5, 11.2 Hz, C15), 3.86-3.82 (1H, m, C26), 3.72-3.61 (4H, C11, C14, C22, C26), 3.70 (3H, s, -OMe), 3.67 (3H, s, -OMe), 3.59-3.54 (1H, m, C5), 3.18 (1H, dd, J = 1.7, 11.7 Hz, C9), 2.49-2.38 (2H, m, C2), 2.35-2.26 (2H, m, C31), 2.21-1.96 (5H, m, C12, C22, C24, C26OH), 2.05 (3H, s, $-OC(O)CH_3$), 1.92-1.86 (2H, m, C12, C14), 1.84-1.76 (2H, m, C32), 1.75-1.70 (1H, m, C6), 1.66-1.44 (5H, C4, C6, C10), 1.33-1.21 (8H, m, C33, C34, C35, C36), 1.15 (3H, s, $-C(CH_3)_2$), 1.01 (3H, s, $-C(CH_3)_2$), 0.91 (3H, s, $-C(CH_3)_2$), 0.88 (3H, t, *J* = 6.7 Hz, C37), 0.82 (3H, s, $-C(CH_3)_2$) ppm.

^{13}C-NMR (125 MHz, $CDCl_3$): δ 172.3, 172.1, 170.9, 167.1, 166.8, 156.5, 151.9, 138.8, 129.9, 119.9, 114.6, 99.0, 85.0, 79.0, 76.6, 74.4, 73.7, 71.6, 68.6, 65.7, 64.7, 51.24, 51.18, 45.0, 44.1, 42.9, 39.9, 37.7, 36.5, 36.1, 35.9, 34.8, 33.9, 31.8, 31.2, 29.9, 29.16, 29.04, 24.83, 24.68, 22.72, 22.63, 21.3, 19.9, 14.2, 13.9 ppm.

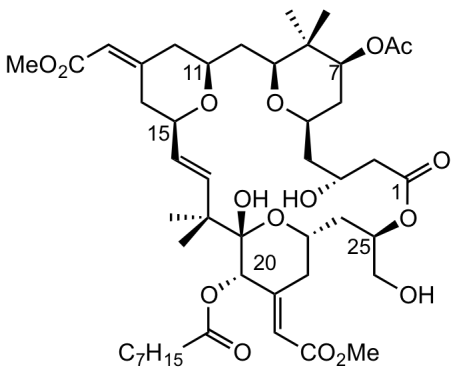
IR (**film**): 3463, 3333, 2927, 2853, 1721, 1652, 1435, 1407, 1378, 1363, 1244, 1154, 1097, 1083, 1048, 1027, 1003, 866, 808, 668 cm^{-1}.

HRMS (TOF MS ES^+): calculated for $C_{46}H_{70}O_{16}Na$: 901.4562; Found: 901.4584.

$[\alpha]_D^{22.8}$ = 25.9 ^{o} (c 0.3, CH_2Cl_2).

$\mathbf{R}_f$ = 0.24 (60% EtOAc: pentane) – one black spot, *p*-anisaldehyde stain.

HPLC: Retention Time = 19.33 min. Method: Semi-prep column; 65% - 100% MeCN in H_2O at 6 mL/min over 30 min.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ppm 9
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